EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-525

EFFECTS OF WHOLE-BODY VX VAPOR EXPOSURE ON LETHALITY IN RATS

Bernard J. Benton
Jeffrey M. McGuire
Douglas R. Sommerville
Paul A. Dabisch
Edward M. Jakubowski, Jr.
Ronald B. Crosier
Robert J. Mioduszewski
Sandra A. Thomson
Kathy L. Crouse

RESEARCH AND TECHNOLOGY DIRECTORATE

Charles L. Crouse

SCIENCE APPLICATIONS
INTERNATIONAL CORPORATION
Abingdon, MD 21009

January 2007

ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-01-2007	Final	May 2005-Nov 2005

4. TITLE AND SUBTITLE
Effects of Whole-Body VX Vapor Exposure on Lethality in Rats

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Benton, Bernard J.; McGuire, Jeffrey M.; Sommerville, Douglas R.; Dabisch, Paul A.; Jakubowski, Edward M., Jr.; Crosier, Ronald B.; Mioduszewski, Robert J.; Thomson, Sandra A.; Crouse, Kathy L. (ECBC), and Crouse, Charles L. (SAIC)

5d. PROJECT NUMBER
206023

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
DIR, ECBC, ATTN: AMSRD-ECB-RT-TT, APG, MD 21010-5424
SAIC, P.O. Box 3465A, Box Hill Corporate Drive, Abingdon, MD 21009

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-525

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Threat Reduction Agency, 8725 John J. Kingman Road, MS 6201, Fort Belvoir, VA 22060-6201

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Much of what is known of the effects of VX on whole animals is derived from studies administering VX percutaneously, subcutaneously, or as an aerosol. Major gaps exist in our understanding of the effects of VX vapor. This study exposed rats to VX vapor in a 1000 L inhalation chamber and established LCT_{50}'s and ECT_{50}'s (for severe effects) at exposure durations of 10, 60, and 240 min. The values were derived from data collected 24 hr post exposure. A potency comparison with GB and GF shows that VX is approximately 4 to 25 times more potent than GB and 5 to 15 times more potent than GF. Gender differences in the LCT_{50} values were not significant. An empirical toxic load model was developed, and the toxic exponent for lethality (n) in the equation $C^n \times T = k$ was determined to be $n = 0.92$. There was a significant depression of AChE of at least 85% at all concentrations tested. Elevated levels of VX-G analog were found in blood plasma at 1 hr post exposure.

15. SUBJECT TERMS
Acetylcholinesterase; Concentration; Toxic load; Lethality; VX; LCT_{50}; Rats; Duration; Inhalation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	91	19b. TELEPHONE NUMBER *(include area code)* (410) 436-2914

Blank

PREFACE

The work described in this report was authorized under Project No. 206023, Low Level Toxicology. The work was started in May 2005 and completed in November 2005. The experimental data are contained in laboratory notebook 05-0069. Raw data and the final report from this study are stored in the Toxicology Archives, Building E-3150, Aberdeen Proving Ground, MD.

In conducting this study, investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institutes of Health Publication No. 86-23, 1985, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, Washington, D.C. These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the U.S. Army Edgewood Chemical and Biological Center (ECBC) Institutional Animal Care and Use Committee (IACUC), which oversees the use of laboratory animals. This project's assigned IACUC Protocol No. 05-366, was approved on 5 May 2005.

All animals were cared for as stated in this research protocol and as specified in the NIH Publication No. 85-23, 1985 (or updates). Records were maintained in official ECBC Notebooks in the Life Sciences Official Archives (Building E-3150) and/or in the Technical Library (Building E-3330). Studies were conducted under, and in compliance with, current GLP standards, and they were reviewed periodically by the QA Coordinator or his designee.

The performance of this study was consistent with the objectives and standards in "Good Laboratory Practices for Non-clinical Laboratory Studies" (21 CFR 58, Food and Drug Administration, U.S. Department of Health and Human Services, April 1988).

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Acknowledgments

The authors wish to thank Dr. Diana Scorpio, Jill Jarvis (Science Applications International Corporation), and Jacqueline Scotto (ECBC) for their support in caring for the animals used in this study. The authors also thank Dennis Johnson (Veterinary Services Team, ECBC) for his guidance with quality assurance issues.

Blank

QUALITY ASSURANCE

This study, conducted as described in Protocol 05-366, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792. The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase Inspected	Date	Reported
Data and Final Report	5 Jun 06	6 Jun 06

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

DENNIS W. JOHNSON
Quality Assurance Coordinator
Toxicology, Aerosol Sciences and
Obscurants Senior Team
Research & Technology Directorate

Blank

CONTENTS

FIGURES

TABLES

Blank

EFFECTS OF WHOLE-BODY VX VAPOR EXPOSURE ON LETHALITY IN RATS

1 INTRODUCTION

O-Ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) is an organophosphorous (OP) compound which has been the subject of much research for over half a century. It is extremely toxic with an equivalent dose of VX being substantially more toxic than related nerve agents such as sarin (GB), cyclosarin (GF), tabun (GA) and soman (GD). Most of what is known of the effects of VX on whole animals is derived from studies administering VX subcutaneously, percutaneously, intravenously or as an inhaled aerosol (Bide and Risk, 2000; Craig, et al., 1977; Gupta, et al., 1991; Rickett, et al., 1986). However, few studies exist in which reliable toxicity estimates in animals have been established for VX administered as a vapor (Hartman, 2002). Contributing to this lack of information is the difficulty in producing stable vapor concentrations in a controlled environment due to the very low vapor pressure of VX (0.00063 mm Hg @ 25°C compared to 2.9 mm Hg @ 25°C for sarin (GB)).

A review of the available literature produced 6 pertinent citations related to whole body VX vapor inhalation exposures in rats. Two deal with the toxicity of chemically neutralized VX (Muse et al., 2002, Manthei et al., 1990), two deal with either aerosolized VX (Bide and Risk, 2000) or O-Ethyl-O'-(2-Diisopropylaminoethyl) Methylphosphonite (QL), a precursor compound to VX (Dimmick et al., 1979). Of the two studies that did generate VX vapor to look at the effects on rats, the first study used the results of rat exposures to miosis levels of VX vapor to propose new limits for human exposures to VX vapor in non-military operations (McNamara et al., 1973). The second study used low concentrations of VX vapor for subacute exposures of rats in an effort to aid in the selection of exposure levels for chronic exposures of laboratory animals to VX vapor (Crook et al., 1983). Neither of these studies had objectives which included the estimation of LCT50's, or exposure durations that approximated what was proposed for this study.

Our first objective was to determine the median lethal concentrations (LC_{50}'s) of VX vapor in rats at three exposure durations. The second objective was to develop an empirical model for predicting VX vapor toxicity for duration times extending beyond our ability to test directly. Our toxic load model was derived from previous work on the dose-response relationships between concentrations of various chemicals and duration of exposure. The relationship, known as Haber's Rule, is described by the equation $C \times T = k$ (Haber, 1924) where C is equal to the atmospheric concentration of the chemical being tested, T is equal to the duration of exposure, and k is a constant for some effect or response. This equation assigns equal importance to concentration and time in determining the response. Thus, the product of C x T would remain constant regardless of the concentration or exposure time (Figure 1). This assumption proved to be inadequate for many chemicals when attempting to describe cumulative toxicity effects. Thus, the equation was modified to better describe the relationship between concentration and exposure time for a given chemical (ten Berge *et al.*, 1986). The equation $C^n \times T = k$ includes the exponent n which is an experimentally determined, chemical specific value which helps describe the non-linear relationship between concentration and duration of exposure (Figure 1). Our third objective was to estimate this n value for lethal levels of VX vapor. Fourth, we were to determine the degree of cholinesterase inhibition in whole blood and VX regeneration in plasma, red blood cells and various tissues. These data provide important

information regarding the relationship between exposure levels, absorption amounts and lethality. VX regeneration data was particularly important because it more directly related to the internal dose the animal was receiving. Our final objective was to determine if the miotic effects of VX vapor exposure and cholinesterase depression were gender dependent.

Whole body vapor exposures were conducted in a 1000 liter dynamic airflow inhalation chamber. Rats were exposed for 10, 60 or 240 minutes. For each duration, five to seven vapor concentrations were used. Baseline values for cholinesterase were established in each rat prior to exposure.

Separate LCT_{50}'s and ECT_{50}'s (severe effects) were established for male and female rats at each exposure duration. The values were derived from data collected 24 hr post exposure. A potency comparison with GB and GF shows that VX is approximately 4-25 times more potent than GB and 5-15 times more potent than GF. Gender differences in the LCT_{50} values were not significant at the 60 and 240 min exposure durations and marginal at 10 min. An empirical toxic load model was developed and the toxic exponent for lethality (n) in the equation $C^n \times t = k$ was determined to be $n = 0.92$ (with 95% confidence limits of 0.90 to 0.94). There was significant depression of AChE activity of at least 85% at all of the concentrations tested. Elevated levels of VX-G-analog (ethyl methylphosphonofluoridate) were found in blood plasma at 1 hr post-exposure and in kidney and lung tissues at 14 days post-exposure. There was no discernible correlation between increasing dosage of VX and levels of VX-G found in blood or tissues.

This study identified experimental effects that could impact operational readiness and serve as a basis for predictions useful for military Operational Risk Management (ORM) decisions.

2 MATERIALS AND METHODS

2.1 Chemicals

O-ethyl-S-[2-(diisopropylamino) ethyl] methylphosphonothiolate (VX or EA 1701) was used for all vapor exposures (ECBC, 2004). The structure of VX is shown in Figure 2. VX was received from the Chemical Transfer Facility at Aberdeen Proving Ground, MD in individually sealed 5- mL ampoules (Lot #VX-U-1243-CTF-N) and certified as chemical agent standard analytical reagent material (CASARM). Seven iterations of a ^{31}P NMR analysis were performed according to an established method (Brickhouse, *et al.*, 1997) to certify the purity of the material as 93.6 ± 0.5 mole percent pure. A high purity grade of triethylphosphate (99.9%; Aldrich Cat. No.: 24,089-3) was used as the internal standard for the VX purity assays. All external standards for VX vapor quantitation were prepared daily with isopropanol (IPA) solvent (Burdick & Jackson Cat. No.: 323-4 purity > 99%).

2.2 Inhalation Chamber

Whole body vapor exposures were conducted in a 1000-liter dynamic airflow inhalation chamber. The Rochester style chamber was hexagonal and constructed of stainless steel with plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.25" H_2O) as recorded by a calibrated magnehelix (Dwyer, Michigan City, IN). Room air was drawn through the exposure chamber (598-783 L/min) and measured at

the chamber outlet with a calibrated thermoanemometer (Alnor model 8565, Skokie, IL). Temperature and humidity were recorded for every exposure.

2.2.1 Vapor Generation

The vapor generation system was located at the chamber inlet and was contained within a stainless steel box maintained under negative pressure. Saturated VX vapor streams (0.13 – 7.04 mg/m^3) were generated by a continuous flow of nitrogen carrier gas (328-1606 sccm/min) through a glass vessel functioning as a multi-pass saturator cell (Glasssblowers Inc., Turnersville, NJ) containing 5 mL of liquid VX (Figure 3). The main body of the saturator cell consisted of a 100-mm long, 25-mm outer diameter (o.d.), cylindrical glass tube with two vertical 7-mm o.d. tubes (inlet, outlet) at each end (Figure 3). The main body of the saturator cell contained a porous, hollow, ceramic cylinder, which served to increase the contact area between the liquid VX and the nitrogen carrier gas by absorbing the liquid VX. The saturator cell was fabricated to allow nitrogen gas to make three passes along the surface of the wetted ceramic cylinder (Alundum® fused alumina, Norton Co., Colorado Springs, CO) before exiting the outlet arm of the saturator cell. The saturator cell body was immersed in a constant temperature bath (Thermo NESLAB, Portsmouth, NH) containing mineral oil so that a combination of nitrogen gas flow rate and temperature could regulate the amount of VX vapor entering the inhalation chamber. The bath was maintained at 50-107.9°C depending upon the required concentration of VX and the outlet arm of the saturator cell was wrapped in heat tape and maintained at 10°C higher than the mineral bath. It was necessary to maintain a continuous flow of VX vapor through the chamber in order to preserve the passivation of the chamber. This allowed for generation and maintenance of stable chamber concentrations.

2.2.2 Sampling System – Sorbent Tubes

The solid sorbent tube sampling system consisted of a 20:35 mesh Tenax-TA fast flow sorbent tube (Dynatherm part number AO-06-2717) and a thermal desorption unit (TDU; ACEM-900, Dynatherm Analytical Instruments, Kelton, PA.) coupled to a gas chromatograph with flame photometric detection (GC/FPD). Samples were drawn from the middle of the exposure chamber by inserting a rod containing a sampling tube through small access ports located on the walls of the chamber. The rod was hooked to a vacuum line that drew a sample through the tube at a rate of 3-5 liters/min for 1-9 minutes depending upon the chamber concentration. Sample flow rates were controlled with calibrated mass flow controllers (Matheson Gas Products, Montgomeryville, PA) and verified before and after sampling with a calibrated flowmeter (DryCal, Bios Int'l, Pompton Plains, NJ) connected in-line with the sample stream. The sample tube was transferred to the TDU and prepared for injection onto a Restek RTX-5 column (15m x 0.32mm x 0.5 μm). Temperature and flow programming within the TDU desorbed VX from the sorbent tube directly onto the GC column. Detection was performed with flame photometric detection in the phosphorous mode.

The sampling system was calibrated by direct injection of external standards onto the sorbent tubes prior to insertion into the TDU and analysis with GC/FPD. In this way, injected VX standards were put through the same sampling scheme as the chamber samples. A linear regression fit ($r^2 = 0.999$) of the standard data was used to calculate the VX concentration of each chamber sample.

Concentration uniformity was checked at several locations throughout the chamber, including areas directly above the animal cages. At higher generated agent concentrations, vacuum pumps were used to draw air through glass fiber, filter pads at high flow rates to test for the presence of aerosols. Analysis of the glass fiber pads required isopropanol desorption and liquid extract injection onto a 20:35 mesh Tenax-TA fast flow sorbent tube. The sorbent tube was thermally desorbed and analyzed by GC/FPD.

2.3 Animal Model

Sexually mature male and female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 180 and 300 gm were used in this study. Upon arrival, the animals were identified by tattoo on the tail and segregated according to sex. Rats were housed individually in plastic shoebox cages. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility (Bldg. E-3150). The animals were quarantined for a minimum of 5 days following their arrival. Ambient conditions were maintained at 70 ± 5°F, 30-70% relative humidity with a 12:12 hour light-dark cycle. Rats were provided with certified laboratory rat chow and filtered house water *ad libitum*, except during exposure. All experiments and procedures were approved by the U.S. Army Edgewood Chemical Biological Center Institutional Animal Care and Use Committee, and conducted in accordance with the requirements of Army Regulation 70-18 and the National Research Council's Guide for the Care and Use of Laboratory Animals.

2.4 Blood and Tissue Sample Collection

Blood samples were drawn from all test rats and used for the cholinesterase inhibition and VX-G analog regeneration assays. Blood draws were done once before exposure, approximately 60-90 min. post exposure, 24 hours and 7 days post exposure. Approximately 1 mL of blood was taken at each draw. In order to promote rapid blood flow and collection of samples, the rats were placed in a "shoebox" type holding cage doubling as a warming pen. The shoebox containing the rats was stacked within a second shoebox containing warm water. The heat from the water elevated the rat's body temperature just enough to promote vessel dilation and increased blood flow. The rats were removed from the warming pen after five minutes and approximately 1/8 inch of their tail was removed using sharp scissors. The tail was gently massaged to promote the collection of blood into Microtainer® tubes (Becton-Dickinson, Franklin Lakes, NJ) containing the anti-coagulant ethylenediaminetetraacetic acid (EDTA). Post collection bleeding was minimal and clotting was facilitated by compression of the incision.

Tissue samples were collected immediately following euthanasia. Tissues collected were eyes, brain, kidney, liver, lung and heart. Following excision, all samples were packaged and frozen in liquid nitrogen.

2.4.1 Cholinesterase (ChE) Inhibition Assays

Approximately 100 uL of blood was collected from a tail snip for use in determination of AChE and BChE activities. All blood samples were collected in EDTA-containing tubes. Assays for AChE and BChE activity were performed on whole blood. Ten uL of whole blood sample was added to a disposable borosilicate glass tube (Chase Scientific Glass, Rockwood, TN) containing 2000 uL of distilled water. Two hundred uL of 0.69 mM phosphate buffer at pH

7.4 (EQM Research, Cincinnati, OH) was then added to each tube. The tubes were vortexed and allowed to sit at room temperature for 20 minutes. Two hundred uL of the sample solution from each tube was transferred to individual wells on a 96-well plate. Twenty-five uL of 30 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) was added to each well. The plate was covered, and incubated at 37°C for 15 minutes. For determination of AChE activity, 25 uL of a solution containing 10mM acetylthiocholine and 200 uM 10-(α-diethylaminopropionyl)-phenothiazine, a specific inhibitor of butyrylcholinesterase (EQM Research, Cincinnati, OH), was added to the appropriate wells of the 96-well plate. For determination of BChE activity, 25 uL of a solution containing 20mM butyrylthiocholine (EQM Research, Cincinnati, OH) was added to the appropriate wells of the 96-well plate. The plate was read at 450 nm and 37°C using a SpectraMax Plus^{384} microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA) for 25 minutes, and analyzed using SoftMax Pro LS version 4.3 software. AChE and BChE activity values were expressed as units of activity per liter of whole blood (U / L).

2.4.2 VX-G Regeneration Assay for Plasma and RBC's

Several days prior and within 1 hour after inhalation exposure, whole blood from VX exposed male and female rats was collected in capped polyethylene tubes that contained EDTA. The samples were centrifuged at 15000 rpm for 5 min to separate the plasma and red blood cell fractions. After separation, the plasma samples were frozen at -20°C until analysis and red blood cell samples were refrigerated at 5°C. The plasma samples were analyzed for VX-G (the G refers to the VX analog ethyl methylphosphonofluoridate) by the addition of acetate buffer and fluoride ion (Jakubowski *et al.*, 2001).

The samples were prepared as follows. To a weighed sample (0.1-0.8 g) of plasma or (0.2-0.3 g) RBC in a 2.0 mL microvial, 1 mL of acetate buffer (pH 3.5), 20 µL/0.1 g sample (for plasma) or 200 µL/0.25 g sample (for RBC) of 6 M potassium fluoride (KF) solution, and 5 µL of $^{2}H_5$-VX-G (200 pg/µL in ethyl acetate) internal standard were added and vortexed. The RBC reaction mixture was centrifuged at 15000 rpm for 1 min to segregate the insoluble components from the solution. These initial reaction solutions were transferred to C_{18} SPE cartridges (200 mg Sep-Pak, Waters Associates, Millipore Corporation, Milford, MA), which were first conditioned with 1 mL ethyl acetate followed with 1 mL isopropanol and finally with 1 mL acetate buffer. The sample microvials were then washed with a mixture of 750 µL acetate buffer and 20 µL/0.1 g sample (for plasma) or 200 µL/0.25 g sample (for RBC) of KF solution. The RBC microvial solution was centrifuged again. The wash solutions were added to the original reaction mixtures on the SPE columns. Fifteen minutes after the original addition of buffer and KF, the combined reaction mixture was allowed to drain through the conditioned SPE column under a gentle vacuum. After complete draining, the SPE column was dried by using a light vacuum to pull air through the column for 3 min. The regenerated VX-G and deuterated internal standard VX-G were eluted with 1 mL ethyl acetate that was collected and dried over anhydrous sodium sulfate. The ethyl acetate was removed from the collection tube and filtered through a 0.2 µm nylon Acrodisc syringe filter (Pall Gelman Laboratory, Ann Arbor, MI) into a GC autosampler vial. The eluent was concentrated to 50-75 µL total volume using a nitrogen stream directed across the sample surface (Techne Sample Concentrator, Techne, Inc., Princeton, N.J.).

The regenerated VX-G was analyzed as follows. Injections of 50 µL (twice) of extract were made by autoinjector into the large volume injector port (Agilent Technologies, model PTV, Wilmington, DE) using the following parameters: initial temperature -30°C, initial time 8.1

min, final temperature 225°C, rate 720°C/min (maximum ballistic heating as listed in the Agilent manual), vent time 8.00 min, vent flow 300 mL/min, purge flow 50 mL/min, purge time 11.7 min.The GC (Agilent Technologies model 6890, Wilmington, DE) column used was a HP-5MS (30 m x 0.32 mm x 1.0 μm film thickness) with a flow rate of 3 mL/min (63 cm/sec). The GC oven program was as follows: initial temperature was 35°C for 12.3 min to 125°C @15°C/min (0 min hold) to 325° C @30° C/min. Mass spectrometric detection (Agilent Technologies model 5973 MSD, Wilmington, DE) was by chemical ionization with ammonia reagent gas in the positive ion mode using the m/z 144/149 ammonia adduct ion ratio (VX-G/$^2H_5$-VX-G) for quantification and the m/z 161 (VX-G) and 166($^2H_5$-VX-G) ions as qualifiers. Linear internal standard calibration curves for VX-G were generated from 10-1000 pg using standards in ethyl acetate. The Agilent software (Enhanced Chemstation Version D.00.00.38, 2001, Agilent Technologies, Wilmington, DE) provided with the mass spectrometer was used to process and analyze the data. The software allowed automatic analysis of the internal standard method based on the analyte area ratios of the peaks at their respective retention times.

2.4.3 VX-G Regeneration Assay for Tissue

Fourteen days after inhalation exposure, tissue samples (brain, heart, lung, liver, kidney, and eye) from VX exposed male and female rats were collected in capped 15 mL polyethylene tubes and immediately flash frozen in liquid nitrogen. None of the tissues were perfused. The samples were immediately transferred to and stored in a refrigerator at -80°C until analysis. The tissue samples were analyzed for VX-G (the G refers to the VX analog ethyl methylphosphonofluoridate) by the addition of acetate buffer and fluoride ion (Jakubowski *et al.*, 2001).

The samples were prepared as follows. To a homogenized (Ultra-Turrax T8, IKA Works, Wilmington, NC) weighed sample (0.1-0.8 g) of tissue in a 15 mL vial, 2 mL of acetate buffer (pH 3.5), 400 μL of 6 M potassium fluoride (KF) solution, 600 μL 1M HCl, and 5 μL of $^2H_5$-VX-G (200 pg/μL in ethyl acetate) internal standard were added and vortexed. The tissue reaction mixture was centrifuged at 4400 rpm (Centra GP8R, Thermo IEC, Waltham, MA), for 10 minutes to segregate the insoluble components from the solution. These initial reaction solutions were transferred to C_{18} SPE cartridges (200 mg Sep-Pak, Waters Associates, Millipore Corporation, Milford, MA), which were first conditioned with 1 mL ethyl acetate followed with 1 mL isopropanol and finally with 1 mL acetate buffer. The sample vials were then washed with a mixture of 1 mL acetate buffer, 200 μL KF, and 300 μL of 1 M HCl solution and vortexed. The tissue vial solution was centrifuged again for 5 minutes. The wash solutions were added to the original reaction mixtures on the SPE columns. The combined reaction mixture was then allowed to drain through the conditioned SPE column under a gentle vacuum. After complete draining, the SPE column was dried by using a light vacuum to pull air through the column for 3 min. The regenerated VX-G and deuterated internal standard VX-G were eluted with 1 mL ethyl acetate that was collected and dried over anhydrous sodium sulfate. The ethyl acetate was removed from the collection tube and filtered through a 0.2 μm nylon Acrodisc syringe filter (Pall Gelman Laboratory, Ann Arbor, MI) into a GC autosampler vial. The eluent was concentrated to 50-100 μL total volume using a nitrogen stream directed across the sample surface (Techne Sample Concentrator, Techne, Inc., Princeton, NJ).

The regenerated VX-G was analyzed as follows. Two 50 μL sample injections of extract were delivered by autoinjector into the large volume injector port (Agilent Technologies, model

PTV, Wilmington, DE) to ensure the entire sample was injected and trapped using the following parameters: initial temperature -30°C, initial time 8.1 min, final temperature 225°C, rate 720°C/min (maximum ballistic heating as listed in the Agilent manual), vent time 8.00 min, vent flow 300 mL/min, purge flow 50 mL/min, purge time 11.7 min. The GC (Agilent Technologies model 6890, Wilmington, DE) column used was a HP-5MS (30 m x 0.32 mm x 1.0 µm film thickness) with a flow rate of 3 mL/min (63 cm/sec). The GC oven program was as follows: initial temperature was 35°C for 12.3 min to 125°C @15°C/min (0 min hold) to 325° C @30° C/min. Mass spectrometric detection (Agilent Technologies model 5973 MSD, Wilmington, DE) was by chemical ionization with ammonia reagent gas in the positive ion mode using the m/z 144/149 ammonia adduct ion ratio (VX-G/$^2H_5$-VX-G) for quantitative analysis.

2.5 Assessment of Toxic Signs

All exposed rats were placed into one of the following four categories of post-exposure toxicity based upon the number and severity of an array of well established indicators of nerve agent toxicity.

Mild Toxicity: Rats were classified as having mild toxicity if they exhibited any or all of the symptoms of miosis, chewing or salivation.

Moderate Toxicity: Rats were classified as having moderate toxicity if they exhibited symptoms of mild toxicity plus moderate tremors and ataxia.

Severe Toxicity: Rats were classified as having severe toxicity if they exhibited symptoms of mild and moderate toxicity plus severe tremors and ataxia and/or prostration, convulsions or gasping.

Lethality: This was determined in each rat via the absence of a heart beat upon palpation

2.6 Decontamination with Reactive Skin Decontaminant Lotion (RSDL)

The chemical name for RSDL is 2,3-butanedione monoximate in a polyethyleneglycol monomethylether vehicle. All exposed rats in Part I of this study (see Section 2.7) were decontaminated with RSDL within 20 to 60 min post-exposure. None of the exposed rats in Part II were decontaminated post-exposure.

When decontaminating exposed rats, individual rats were placed in a poly vinyl chloride (PVC) tube 2 inches in diameter and 10 inches in length. The tubes were capped at both ends and perforated with ¼ inch drain holes. Rats were completely immersed one at a time in the RSDL. The rats remained in the tubes and in contact with the RSDL for 10 min following immersion in RSDL. After 10 min, the rats were immersed in a warm water rinse to remove most of the RSDL. This procedure was found to eliminate all traces of VX from the rat's body. Following decontamination, rats were considered safe to handle and were used to collect blood and tissue samples over a two week period.

2.7 Experimental Design

Rats were exposed to VX vapor for durations of 10, 60 or 240 minutes. In Part I, five to seven different concentrations of VX were tested within each duration group. The concentration values used were chosen to best investigate the lethality dose response curve from approximately

0 to 5% lethality up to 90% lethality. Each exposure group consisted of 10 male and 10 female rats. For each exposure, 5 males and 5 females were placed into each of two compartmentalized stainless steel cages (20" x 14" x 4") with each rat occupying a separate compartment (4" x 7" x 4"). Both of these steel cages were placed on the floor of the exposure chamber prior to the introduction of VX vapor. For each exposure, 1 male and 1 female control rats were placed in a separate "clean" chamber and exposed to air only. Following exposure, the two exposure cages were removed from the chamber. One cage was chosen at random and survivors were removed from the cage and underwent assessment of toxic signs and the decontamination process first. Decontamination for this group now referred to as decontamination group 1 (D1) was started approximately 20 min. post-exposure and took about 30 min. for completion (see section "Decontamination with Reactive Skin Decontaminant Lotion (RSDL)"). Following completion of decontamination of D1 survivors, rats in the 2nd steel exposure cage underwent the same process of removal, toxic sign evaluation and decontamination. This 2nd group is referred to as decontamination group 2 (D2). The decontamination process for D2 was started approximately 60 min. post-exposure and took approximately 30 min. for completion. The only difference between D1 and D2 was the additional 40 min. the survivors in D2 remained in the steel exposure cage while waiting to be decontaminated. Decon groups D1 and D2 were equally represented in each VX vapor chamber run of a particular vapor concentration, with 5 males and 5 females in each decon group. Blood and tissue samples were collected from decontaminated survivors in D1 and D2 over a 2 week period after exposure. For D1 and D2 survivors clinical signs of exposure were monitored once daily for 48 hr post-exposure. After 14 days post-exposure, surviving rats from D1 and D2 were euthanized.

Part II used the same three exposure durations (10, 60 and 240 minutes) used in Part I, but only five different VX vapor concentrations per duration were used (versus the five to seven values used in Part I). As in Part I, the concentration values used were chosen to best investigate the lethality dose response curve from about 0 to 5% lethality up to about 90% lethality. These concentrations were different from those used in Part I for decon groups D1 and D2. Each exposure group in Part II consisted of 10 male and 10 female rats (which were exposed in two steel exposure cages with 5 males and 5 females in each cage). However, the survivors from Part II were not decontaminated, and they were euthanized 24 hr post-exposure. The rats in Part II will be referred to as the no-decontamination group (ND). For the ND group survivors, clinical signs of toxicity were monitored immediately after exposure and again at 24 hr post-exposure. All euthanasia was done in accordance with the *2000 Report of the AVMA Panel on Euthanasia*.

For all exposure groups, the t_{99} (time to attain 99% of the equilibrium concentration within the chamber) ranged from 5.6-7.7 minutes. Physical parameters monitored during exposure included chamber airflow, nitrogen flow rate through the saturator cell, chamber room temperature and relative humidity. Following exposure, the chamber was purged with air for 10 minutes prior to removing the rats.

2.8 Data Analysis

Minitab®, Version 14 (Minitab, Inc., State College, PA) was used for all statistical analyses. Printouts of the Minitab® analyzes are listed in the Appendix.

Binary and ordinal logistic regressions (with a normit link function) (Finney, 1971; Agresti, 1990; Fox, 1997) were used to fit both binary and ordinal responses observed in this

study. They were used to investigate how the probability of effect varied as a function of several parameters (vapor concentration, exposure duration, gender, use of decontamination solution on the rat's skin post-exposure, *etc.*). This approach has been used successfully in several previous mammalian CW agent toxicity studies (Anthony, *et al.*, 2003; Hulet, *et al.*, in preparation; Mioduszewski, *et al.*, 2002a, 2002b; Sommerville, 2004; Whalley, *et al.*, 2004). Several different mathematical models were used (depending on the circumstances), and these models can be divided into two broad categories: those with vapor concentration, C, and exposure duration, T, are analyzed as a combined term, CT (or a dosage), and those models in which the effects of C and T are accounted for separately.

The models of the first category (C and T combined into a dosage term) were used to calculate median effective dosages (for moderate and severe effects, and lethality) for each gender-exposure duration dataset within Parts I (exposures of decon groups D1 and D2) and II (exposure of ND decon group). In addition, probit slope estimates were calculated for each individual dataset and for various combinations of datasets (all the datasets of Part I, all the datasets of Part II, and the datasets of Parts I and II combined). Possible effects due to gender and decontamination paradigm were investigated as well. The models of the first category were:

$$Y_N = (Y_P - 5) = k_0 + k_{CT}(\log_{10} CT) + k_{DG}\, Dgroup + k_S\, Sex \qquad [1]$$

$$Y_N = k_0 + k_{CT}(\log_{10} CT) + \sum_{j=1}^{3} k_{D,j}\, Decon_j + k_S\, Sex + \sum_{j=1}^{3} k_{DS,j}\, Decon_j \times Sex$$

$$\text{with } k_{D,1} = k_{DS,1} = \text{zero} \qquad [2]$$

$$Y_N = k_0 + k_{CT}(\log_{10} CT) + \sum_{i=1}^{N} k_{Set,i}\, Set_i + k_s\, Sex$$

$$\text{with } k_{Set,1} = \text{zero} \qquad [3]$$

where Y_N is a normit; Y_P is a probit; the k's are fitted coefficients; CT is the dosage; Dgroup is coded 1 for decon group D1 and -1 for decon group D2 (and k_D and k_{DS} both equal zero for gender-exposure duration datasets in Part II); $Decon_j$ is an indicator variable for the three different decontamination scenarios (D1, D2 and ND), with $Decon_j$ equaling one for the j^{th} decon group and zero otherwise (see also Table 1); Sex is coded 1 for males and -1 for females; and Set_i is an indicator variable (with N being the number of datasets being analyzed), with Set_i equaling one for the i^{th} dataset and zero otherwise.

The intercept, k_0, is dependent on the toxicological endpoint, and in the case of a binary response, k_0 serves as the traditional model intercept. The fitted coefficient, k_{CT}, is the estimate for the probit slope. Y_N equals-1, 0 and 1, at the 16, 50 and 84 percent response levels, respectively. When using Equations [1] to [2] to model an ordinal response, the following scoring system was used: mild effects or lesser effects (Score = 1), moderate effects (Score = 2), severe effects (Score = 3) and lethality (Score = 4)(see Section 2.5). All scores are defined on a 24 hour basis.

The models of the second category (C and T expressed as separate terms) were used to calculate the time dependence of the VX toxicity and to more fully investigate the influence of the decontamination scenario on VX toxicity. The models of the second category were:

$$Y_N = k_0 + k_C(\log_{10} C) + k_T(\log_{10} T) + k_{DG}\, Dgroup + k_S\, Sex \qquad [4]$$

$$Y_N = k_0 + k_C(\log_{10} C) + k_T(\log_{10} T) + \sum_{j=1}^{3} k_{D,j}\, Decon_j + \sum_{j=1}^{3} k_{DT,j}\, Decon_j \text{ x } (\log_{10} T)$$

$$\text{with } k_{D,1} = k_{DT,1} = \text{zero} \qquad [5]$$

$$Y_N = k_0 + k_{CT}(\log_{10} CT) + \sum_{i=1}^{3} (Time)_i \left(k_{Time,i} + k_{\bar{D},i}\,\bar{D} + k_{\bar{R},i}\,\bar{R}\right)$$

$$\text{with } k_{Time,1} = \text{zero} \qquad [6]$$

where all terms are defined as before, with the addition of *Time* as a three-level factor representation for exposure duration (versus treating it as a covariate in Equations [4] and [5]), $\bar{D}$ and $\bar{R}$ are contrasts for estimating the effect on toxicity from the decontamination scenario and the period of removal of the animals (post-exposure) from the exposure cage. The values for these contrasts are shown in Table 1.

The contrast, $\bar{D}$, is used to gauge the effect of decontaminating the rat's skin (post-exposure) upon the prompt removal of the rats from the exposure cage at the conclusion of the experiment. The other contrast, $\bar{R}$, is used to gauge the difference between prompt versus delayed removal of rats from the exposure cage, but only for those rats that subsequently undergo decontamination upon removal. All rats in Part II (decon group ND) were promptly removed from the exposure cage; so, it is not possible to compare the difference between prompt versus delayed removal of rats in those cases where rats do <u>not</u> undergo decontamination. Also, the existence of any interaction between the post-exposure use/non-use of decontamination and removal time (prompt versus delayed) cannot be determined from the three pairs that were investigated (prompt removal—decon, prompt removal—no decon, and delayed removal—decon). Thus, care must be exercised in interpreting the model fits for these contrasts: significant $\bar{D}$ values only apply to rats that are promptly removed, and significant $\bar{R}$ values only apply to rats that are eventually decontaminated within one hour post-exposure.

The ratio (k_C / k_T) equals the toxic load exponent, n, or in the case of Equation [5], the toxic load exponent equals ($k_C / \{k_T + k_{DTj}\}$). If this ratio is not different (with statistical significance) from one, then Haber's Rule (Haber, 1924) is appropriate for modeling the toxicity (Figure 1). Otherwise, the classic toxic load model (C^nT) is the proper approach (ten Berge, *et al.*, 1986; Sommerville, *et al.*, 2006) assuming there is no significant curvature in the experimental data used to fit the model.

3 RESULTS

This study focused on collecting sufficient quantal data to estimate median effective dosages for severe effects and lethality in rats exposed to VX vapor for 10, 60 or 240 minutes. Subsequently, these data were used to formulate a multifactor model to predict dose-response relationships and the probability of incurring VX vapor induced effects as a function of exposure concentration and duration. In addition, sufficient data was also collected to estimate the effects of gender, and rat decontamination and removal scenario (post-exposure) on VX toxicity, as well as their potential interactions with vapor concentration and duration. By exposing groups of 20 rats each to five to seven different concentrations of VX vapor per exposure duration, we were able to establish median effective dosages for moderate effects (in some cases), severe effects and lethality for both male and female rats at each of the exposure durations within each of the three decon groups (D1, D2 and ND). The blood samples collected pre- and post-exposure were analyzed for dosimetric correlations between exposure dosage, whole blood cholinesterase activity and the levels of VX-G found in blood plasma, rbc's and various tissues. The results of the data analysis are described below.

3.1 Median Effective Dosages and Probit Slopes for Lethal and Sub-Lethal Effects

Estimates for the median effective dosages for severe effects and lethality (using Equation [1]) for each of the individual gender/exposure duration/decon group (D1, D2 and ND) datasets are listed in Table 2, along with their associated 95% fiducial limits. These values are also shown in Figures 4 and 5.

A possible outlier was identified (via analysis of the standardized Pearson residuals) during the course of this analysis: Part II quantal data from a 10 minute exposure to vapor concentration of 5.5 mg/m^3. Only four rats (two female and two male) died during this run, whereas the model fitted using Equation [1] predicts that twelve rats should have died (five female and seven male). The next lowest concentration (5.2 mg/m^3) produced 16 deaths, and the next highest (6.4 mg/m^3) produced 17 deaths. Also, there is statistically significant model lack of fit when the outlier is included in the dataset, whereas its removal produces no lack of model fit. Thus, values for median effective dosages, probit slopes and severe to lethal median effective dosages are reported both with and without this outlier in Tables 2-4.

3.2 Probit Slopes for Lethal and Sub-Lethal Effects

The probit slopes values associated with the median effective dosages mentioned in the previous section (the fitted k_{CT} value from Equation [1]) are listed in Table 3, along with their standard errors. These values ranged from 8.3 to 23.1. Within a particular dataset, the probit slopes for severe effects and lethality are assumed to equal each other (a model requirement for ordinal regression using MINITAB®). It was found for the rats in Part I that there was no statistically significant difference between the decon groups D1 and D2 with respect to probit slope values within any gender/exposure duration dataset (*ex.* for 10 minute exposure of female rats in Part I, the same probit slope value is reported for both decon groups D1 and D2). Probit slopes were also calculated (using both Equation [3] with ordinal regression and the weighted average of probit slopes for individual gender/duration/decon group datasets) for Part I (all durations combined), Part II (all duration combined), and Parts I and II combined. These values are also listed in Table 3. The probit slope for Parts I and II combined using ordinal regression

equals either 10.3 (with 95% CL of 9.0 to 11.8) with the previously mentioned outlier or 11.2 (with 95% CL of 9.8 to 12.6) without. The values are slightly higher when using the weighted average approach for Parts I and II combined, equaling either 11.2 (with 95% CL of 9.8 to 12.6) with the previously mentioned outlier or 12.3 (with 95% CL of 10.9 to 13.7) without.

3.3 Relationship between Median Effective Dosages for Lethal and Sub-Lethal Effects

The values of the ratios of median effective dosages (severe effect/ lethality) for each of the individual gender/exposure duration/group were calculated (using Equation [1] with ordinal regression) are listed in Table 4 (with 95% confidence limits), as well as plotted in Figure 6. The 10 minute ratio values shown are those based on calculations without the outlier. The ratio values range from 0.64 to 0.85. Dosage ratios were also calculated (using Equation [3] with ordinal regression) for Part I (all durations from decon groups D1 and D2 combined), Part II (all duration combined for decon group ND), and Parts I and II combined. These values are also listed in Table 4. The dosage ratio for Parts I and II combined equals either 0.727 (with 95% CL of 0.697 to 0.759) with the outlier or 0.739 (with 95% CL of 0.711 to 0.769).

A weighted linear regression analysis was performed on the 12 ratio values shown in Figure 6, using the inverse of the squares of standard errors of the individual ratio estimates as the weights. The least square fits are shown in the figure. It was found that the log(ratio) values were dependent on the log$T$, and there was a statistically significant interaction between log$T$ and presence of decontamination. There was no significant gender effect. The presence or absence of the outlier did not affect the statistical significance (or lack of significance) of these factors. The R-sq for the LSQ fit was 84.3%. The practical effect of this fit is that for group ND (no decontamination post-exposure), the ratio value is essentially constant at roughly 0.75; while for groups D1 and D2, the ratio increases in value from 0.67 to 0.85 as the exposure duration increases from 10 to 240 minutes.

3.4 Gender Effects on Toxicity

Equations [1] to [3] were used to investigate whether any statistically significant effects on toxicity due to gender existed. Ordinal regression (using Equation [1]) was performed on each exposure duration dataset within Parts I and II, and it was found that a statistically significant gender effect was only present in two datasets: the 10 minute exposures from Parts I and II. For the other four datasets (the 60 and 240 minute exposures of Parts I and II), no significant gender effect was detected. For the Part I 10 minute exposure, the ratio of median effective dosages (male to female) was found to equal 1.10, with approximate 95% confidence limits of (1.02 to 1.20). For the Part II 10 minute exposure, the dosage ratio equals either 0.89 (with the 5.5 mg/m^3 outlier) or 0.88 (without the outlier), with approximate 95% confidence limits of (0.79 to 1.0) and (0.80 to 0.97), respectively. Thus, the males were more resistant than the females to VX vapor in Part I (decon groups D1 and D2) and less resistant in Part II (group ND). This interaction between gender and decon group (D1, D2 and ND) was investigated further by combining the 10 minute exposure data from Parts I and II together into a larger dataset and then performing an ordinal regression using Equation [2]. It was determined that gender was statistically significant for decon groups D1 (males more resistant) and ND (males less resistant). For decon group D2, there was no significant gender difference.

3.5 Decontamination Scenario Effects on Toxicity

Equation [1] was used to compare decon groups D1 and D2 from Part I, and it was found that the difference between these two groups was constant with respect to exposure duration, with median effective dosage ratios (D1 to D2) ranging from 1.14 to 1.15. However, the relationship between these two groups and group ND is more complex. The difference between Part I (decon groups D1 and D2) and Part II (decon group ND) varies with respect to exposure duration, with the greatest difference occurring at 10 minutes and the smallest at 240 minutes. The relative rankings of median effective dosages for the three decon groups are as follows (based on ordinal regression using Equations [3] and [6]): at 10 minutes (D1 > D2 > ND); at 60 minutes (D1 > D2 and ND); and at 240 minutes (D1 > ND > D2). The differences between the three groups (D1, D2 and ND) are statistically significant, except at 60 minutes where there is no difference between D2 and ND.

The contrast $\bar{D}$ (for comparison of decontamination versus non-decontamination upon the rats' immediate removal from the exposure cage) is statistically significant only for 10 and 60 minutes, while the contrast $\bar{R}$ (for comparison of prompt versus delayed removal from the exposure cage for rats that are decontaminated) is significant only at 240 minutes. Furthermore, there is a significant interaction between Sex and $\bar{D}$ at 10 minutes (though not at 60 minutes). The ratio of median effective dosages ($\bar{D}$ = 1 / $\bar{D}$ = -1) for 10 minutes was either 1.32 with 95% confidence limits of 1.22 to 1.46 (with outlier), or 1.41 with 95% confidence limits of 1.29 to 1.54 (w/o outlier). For 60 minutes, the ratio is equal to 1.19 with 95% confidence limits of 1.09 to 1.30. The ratio of median effective dosages ($\bar{R}$ = 1 / $\bar{R}$ = -1) for 240 minutes was 1.20 with 95% confidence limits of 1.10 to 1.30. Thus, decontamination upon immediate removal only has an effect for the short exposure durations, while the promptness of removal for decontaminated rats was only significant for the longest duration (240 minutes).

3.6 Analysis of Time-Dependence of Toxicity

The effect of exposure duration on VX inhalation toxicity was investigated via ordinal regression using Equation [4]. The following normit fits were obtained for the Part I dataset (decon groups D1 and D2):

$$Y_N\{\text{Score} = 2\} = (-16.4854) + (9.9491)(\log_{10} C) + (11.2176)(\log_{10} T) + (-0.3740)\,Dgroup \quad [7]$$

$$Y_N\{3\} = (-17.6141) + (9.9491)(\log_{10} C) + (11.2176)(\log_{10} T) + (-0.3740)\,Dgroup \quad [8]$$

$$Y_N\{4\} = (-19.0545) + (9.9491)(\log_{10} C) + (11.2176)(\log_{10} T) + (-0.3740)\,Dgroup \quad [9]$$

In Part I, all the exposed rats had mild effects or greater, so it was only possible to calculate toxic load fits for moderate effects (Score = 2), severe effects (Score =3), and lethality (Score = 4). Equations [7] to [9] are on a one day lethality basis. Thus, the toxic load exponent (n) was found to equal (9.949 / 11.218) or 0.89, with 95% confidence limits of 0.87 to 0.90. Decon group (Dgroup) was found to be statistically significant with the Part II rats, with D1 rats being more resistant by a factor of 1.19.

The following normit fits were obtained for the Part II dataset (group ND):

$$Y_N\{3\} = (-16.0581) + (9.7126)(\log_{10} C) + (10.5544)(\log_{10} T) + (0.1247)\,Sex \quad [10]$$

$$Y_N\{4\} = (-17.4070) + (9.7126)(\log_{10} C) + (10.5544)(\log_{10} T) + (0.1247)\,Sex \quad [11]$$

In Part II, only two rats had score values of 1 (mild effects), which was not enough to accurately calculate a toxic load fit for mild effects. Thus, the scores for these two rats were changed from 1 to 2 when calculating the toxic load fits for Part II. When the previously mentioned outlier is dropped from the analysis, the following fits are obtained:

$$Y_N\{3\} = (-19.3389) + (12.0642)(\log_{10} C) + (12.7200)(\log_{10} T) + (0.1423)\,Sex \quad [12]$$

$$Y_N\{4\} = (-20.7827) + (12.0642)(\log_{10} C) + (12.7200)(\log_{10} T) + (0.1423)\,Sex \quad [13]$$

The toxic load exponent for Part II equals either 0.92 (95% CL of 0.90 to 0.94) with the outlier or 0.95 (95% CL of 0.93 to 0.97) without the outlier. Gender (*Sex*) was found to be statistically significant with the Part II rats, with the male rats being less resistant by a factor of 0.95.

Plots of the toxic load fits from Equations [8], [9], [12] and [13] are shown in Figures 4 and 5. All of the above toxic load exponent values are statistically different from one (since none of the 95% confidence intervals overlap a value of one). Therefore, a toxic load model better describes the time-dependence of the probability of toxic effects than does Haber's Rule. For Part I rats, the toxic load exponent was independent of both decon group (D1 versus D2) and gender, and for Part II rats, the exponent was independent of gender.

Potential lack of fit for the toxic load model was tested for by adding the term $(\log_{10}T)^2$ to Equation [4] to test for curvature with respect to exposure duration. This term was found to be not statistically significant.

3.7 Blood ChE Response

Figure 7 is a comparison between groups D1 and D2 of the whole-blood AChE activity at 1 hr post-exposure for several concentrations of VX vapor at each exposure duration. There was no significant difference in AChE activity between D1 and D2 at any of the measured concentrations and exposure durations. Similarly, there were no significant differences in AChE activity between D1 and D2 at any of the VX vapor concentrations and exposure durations at 24 hr or 7 days (Table 5).

Figure 8 illustrates the AChE activity at 1 hr post-exposure. Male and female rats were grouped together since no significant differences were found between males and females. (A) For 10-minute exposures, all concentrations of VX investigated depressed AChE by approximately 90%. No difference existed between the concentrations. (B) For 60-minute exposures, all concentrations of VX investigated depressed AChE activity. However, unlike the 10-minute exposures, the degree of depression was concentration-dependent. (C) For 240-minute exposures, all concentrations of VX investigated depressed AChE activity. The degree of

depression was again concentration-dependent, with higher concentrations producing significantly more depression.

Figure 9 illustrates the AChE activity at 24 hours post-exposure. Male and female rats were grouped since no significant differences were found between males and females. (A) For 10-minute exposures, all concentrations of VX investigated resulted in a depression of AChE activity at 24 hours post exposure. Several concentrations produced more inhibition of AChE than others; however, this difference was small, and was not concentration-dependent. (B) For 60-minute exposures, all concentrations of VX investigated resulted in a depression of AChE activity at 24 hours post exposure. No difference existed between the concentrations. (C) For 240-minute exposures, all concentrations of VX investigated resulted in a depression of AChE activity at 24 hours post exposure. The degree of depression was again concentration-dependent, with higher concentrations producing significantly more depression.

Figure 10 illustrates the AChE activity at 7 days post-exposure. Male and female rats were grouped since no significant differences were found between males and females. (A) For 10-minute exposures, animals had recovered to approximately 80% of baseline at all concentrations of VX investigated by 7 days post-exposure. (B) For 60-minute exposures, animals had recovered to approximately 80% of baseline at all concentrations of VX investigated by 7 days post-exposure. (C) For 240-minute exposures, animals had recovered to 50-80% of baseline at all concentrations of VX investigated by 7 days post-exposure.

The effect of VX vapor on whole blood BChE activity was not able to be determined from the current data due to a large degree of variability in the data. However, differences in baseline BChE activity between males and females was observed, with females (333 ± 7 U/L) having a significantly higher level of activity than males (288 ± 7 U/L).

3.8 Fluoride Ion Generated VX-G Analog in Blood Plasma and RBC's

Figures 11-13 summarize the results of the VX-G (ethyl methylphosphonofluoridate) analog assay of the blood plasma and rbc's from D1 and D2 exposed rats. At each of the three exposure durations, blood plasma and rbc values for only the lowest dosages are presented. Also, for Figures 11-13, VX-G values are shown for only the 1 hr and 24 hr sampling periods. No VX-G data is shown for the 7 day post-exposure sampling period because the amounts of VX-G present in the plasma and rbc's was usually below detectable limits.

In general, more VX-G was found in the plasma rather than the rbc fraction of whole blood. In the plasma, the largest amount of VX-G was present at the 1 hr post-exposure sampling period. For both groups, the elevated plasma levels of VX-G at 1 hr post-exposure dropped considerably by 24 hr post-exposure. There was a smaller percent reduction of VX-G in the rbc fraction between 1 hr and 24 hr post-exposure (Figure 11). There were no significant differences in plasma or rbc values for VX-G between the D1 and D2 groups.

Figures 14-16 summarize the plasma and rbc levels of VX-G at each of the three exposure durations for the rats that were not decontaminated (ND group). This group was only sampled at 24 hr post-exposure. Figures 11 and 14 (10 min exposure durations) illustrate across a range of VX dosages, the larger concentrations of VX-G found in the plasma and rbc fractions of the ND group sampled at 24 hr. In Figure 11, the range of VX-G (both D1 and D2) across all of the concentrations shown at 24 hr was 0.25$\pm$ 0.03 to 0.49$\pm$ 0.05 ng/g for plasma and 0.17$\pm$0.02 to 0.24$\pm$0.02 ng/g for rbc's. The range of VX-G for the ND group shown in Figure 14 was 0.55$\pm$0.03 to 1.14$\pm$0.19 ng/g for plasma and 0.56$\pm$0.17 to 0.80$\pm$0.17 ng/g for the rbc

fraction. The amount of VX-G present in both the plasma and rbc fractions of the ND group is more than twice that of either D1 or D2. For the 60 min exposure duration (Figures 12 and 15) a comparison of rbc levels is not possible but the plasma levels for D1 and D2 (Figure 12) range from 0.51±0.14 to 0.60±0.09 ng/g compared to 0.50±0.05 to 1.85±0.36 ng/g for the ND group (Figure 15). For the 240 min exposure duration (Figures 13 and 16), there is no rbc values for 24 hr and only one dosage shown for D1 and D2 in Figure 13. The range for the 24 hr plasma values for D1 and D2 in Figure 13 was 0.77±0.08 to 1.43±0.46 ng/g and the 24 hr range for the ND group in Figure 16 is 0.46±0.07 to 1.61±0.40 ng/g of VX-G. It appears that with increasing exposure duration, there is more overlap in the ranges of plasma VX-G between decontaminated and non-decontaminated rats.

Given the limited number of rats sampled for the regeneration assay (Figures 11-16) in this study, there was no discernible pattern of increasing amounts of plasma/rbc VX-G with increasing dosage of VX vapor.

3.9 Fluoride Ion Generated VX-G Analog in Tissues

Fourteen days post-exposure, exposed rats were euthanized and various tissues were excised and frozen in liquid nitrogen. The tissues sampled were brain, lung, liver, kidney, eye and heart. Figure 17 is a representative example of the relative amount of VX-G found in each of the tissues. Regardless of the exposure duration or dosage, the kidney and lung consistently ranked first and second, respectively, as the tissues containing the most regenerated VX-G. There were significant differences between D1 and D2 in the amounts of VX-G in the kidney and lung (t-test, $p<.05$).

4 DISCUSSION

The inhalation toxicity of VX vapor in rats (via whole-body exposures) can be characterized as having steep dose-response curves: severity of effect versus dosage (as represented by a high ratio (0.75) of severe effects to lethal median effective dosages), and percent affected individuals versus dosage (with a probit slope of 10 to 11). Furthermore, based upon the empirical evidence of the toxic load exponent (n) value for the rats that were not decontaminated post-exposure (decon group ND) ($n = 0.92$ or 0.95), VX vapor becomes more toxic as a function of increasing exposure duration. Since these values for n are statistically different from one, the toxic load model better describes the time-dependence of the probability of toxic effects than does Haber's Rule.

In fact, for all three decon groups (D1, D2 and ND), the toxic load model is a better predictor than Haber's Rule, but the fit for the group ND comes closest to Haber's Rule. The difference between the LCT_{50} predictions of the toxic load fit for group ND (with $n = 0.95$) at 10 minutes and 240 minutes (46.6 and 39.2 mg-min/m^3, respectively) are only a factor of 1.19 apart. Thus, the error in assuming Haber's Rule over the duration range of 10 to 240 minutes would not be that great—the geometric average (43) of 46.6 and 39.2 would only be off by a factor of 1.1 (or 0.595) at the most. However, the error will steadily increase if one extrapolates using Haber's Rule beyond the range of 10 to 240 minutes.

The relative constant toxicity of VX vapor exposure (described above) is in contrast to the whole-body exposures of rats to GB (Mioduszewski, *et al.*, 2002a, 2002b) and GF (Anthony,

et al., 2004) vapor, which have toxic load exponent values of 1.66 and 1.24, respectively. A comparison of the present study with the results from the GB and GF lethality studies are shown in Table 6. Some of the information for Table 6 comes from Sommerville, 2004. The individual LCT_{50} values from these studies are compared to those of the present study in Figure 18.

Comparison of the GB, GF and VX rat inhalation toxicity data shows that the potency ratio between the pairs, (VX versus GB) and (VX versus GF), is not constant with respect to exposure duration. In both cases, the potency of VX relative to the other two agents increases with duration. At short durations, the ranking of potency is VX > GB (4 to 6x), VX > GF (5 to 7x), but at the longer durations, GB and GF swap places to produce the ranking of VX > GF (13 to 15x), VX > GB (21 to 25x). For 60 minute exposure durations in the rat, GB and GF are equally potent, but VX is about 9 to 11 times more potent than either of these agents. The gender differences are also more pronounced for GB and GF than for VX (which has a very small though statistically significant difference at 10 minutes).

The time-dependency of VX toxicity is dependent on how the rats were handled post-exposure, with decontamination (either after prompt or delayed removal from the exposure cage) decreasing the value of the toxic load exponent from 0.95 (the value for non-decontaminated rats) to 0.89. As illustrated in Figures 4 and 5, the biggest difference between the decontaminated rats (groups D1 and D2) and the non-decontaminated rats (group ND) occurs at 10 minutes. At the longer durations, decontamination (post-exposure) is less beneficial, and in fact at 240 minutes, group D2 (delayed removal and decontamination) has a lower LCT_{50} value than group ND (prompt removal and no decontamination). Thus, for exposures to VX vapor concentrations over long exposure durations, decontamination only has value if the rats were promptly removed from the contaminated environment.

Since the primary objective of this study was to establish LCT_{50}'s for VX vapor, certain concessions were made with regards to collecting blood samples for the AChE and VX-G Regeneration assays. In particular, no blood samples were drawn from any rats that died during the exposure or rats too sick to risk taking a blood sample lest they die from stress factors not associated directly with the VX exposure. Therefore, the results of the AChE and VX-G assays presented in Figures 7-10 (AChE) and Figures 11-16 (VX-G) do not include the higher dosages due to the small number of blood samples drawn from rats exposed to these dosages. Although the limited data sets prevent us from being too specific, several observations regarding the results of the AChE and VX-G Regeneration assays bear mentioning.

There were no significant differences in AChE activity attributable to gender or between D1 and D2 at any of the dosages that were tested (Figure 7). At 1 hr and 24 hr post-exposure, all dosages tested depressed whole-blood AChE activity a minimum of 85% (Figures 8,9). At 7 days post-exposure, AChE activity had rebounded to between 40 and 90% of control across all dosages. No AChE data was obtained for the ND group at 24 hr, therefore comparisons with D1 and D2 were not possible. It would have been unlikely to see significant additional AChE depression beyond 85-90% as was seen in D1 and D2. This is because of the limited effectiveness of RSDL in preventing further AChE depression from percutaneous absorption of VX following exposure durations of 60 and 240 min. Lundy, *et al.*, 2004 investigated the effectiveness of RSDL at arresting the progression of ChE depression and other toxic symptoms of percutaneously applied VX in swine. They found that ChE depression following site application of VX on the ear, was essentially complete (approximately 90% depression) after 45 min. RSDL was most effective at preventing the progression of ChE depression when it was applied to the exposure site within 15 min post-VX exposure. It's effectiveness at preventing

further progression to toxic symptoms such as apnea declined rapidly after 15 min. This time-line for the effectiveness of RSDL is consistent with the results of our study in that the effects of RSDL were most apparent in the LCT_{50} values calculated for D1, D2 and ND groups for 10 min exposures. For our 10 min exposures approximately 30 min elapsed from the start of VX exposure until application of RSDL.

The results of the VX-G Regeneration assay showed more VX-G was found in the plasma rather than rbc fraction of the whole-blood. This was expected since a larger number of potential binding sites (cholinesterases and other proteins) are found in rodent plasma. Within the plasma fraction, the largest amounts of VX-G were found at the 1 hr post-exposure sample time but these elevated levels decreased dramatically by 24 hr post-exposure (Figures 11-13). There was a much smaller reduction in the levels of rbc VX-G over the same time period. The six tissues sampled for VX-G in this study, were harvested 14 days post-exposure. Of the 6 tissues sampled at 14 days post-exposure, the kidney and lung tissue consistently contained the most regenerated VX-G (Figure 17). These elevated levels in the kidney and lung are consistent with the results of another report (Martin, 1991) that found the highest levels of H^3Soman at 24 hr post-exposure were in the lungs, heart and kidneys, respectively, of mice that were given the soman via two different routes (intramuscular and inhalation). It is certain that the relative distribution amounts in the tissues vary with the post-exposure sample time. In fact, Whalley, *et al.*, 2005 using guinea pigs, found a much different distribution of GB in the same 6 tissue types sampled at 2 hr and 24 hr post-inhalation exposure. At 2 hr post-exposure, the lung, eye and liver, respectively, contained the most GB whereas at 24 hr, the eye, lung and kidney, respectively, contained the most GB.

Finally, there were no discernible trends between increasing dose of VX vapor and increasing amounts of VX-G in the plasma/rbc fractions of whole-blood. Any correlations that might exist were not identifiable because rats exposed to the largest dosages were not sampled for the VX-G or ChE assays. In addition, there were small numbers of n for the rats exposed to even the lower dosages of VX vapor.

5 CONCLUSIONS

This study filled some of the gaps in our understanding of the toxic effects of severe/lethal-level VX vapor exposures. ECT_{50}'s (severe), LCT_{50}'s, a toxic load exponent, blood AChE inhibition and VX-G levels in blood and tissue were calculated for rats exposed for 10, 60 or 240 min. Ordinal regression was used to develop empirical toxic load models ($C^{0.92}xT=k$ for lethality) to describe the effects of VX vapor dosage over time. Although the toxic load model is a better predictor than Haber's Rule, the fit for the non-decontaminated rats (decon group ND) comes closest to Haber's Rule. Gender differences to the effects of VX vapor in this study were only marginally significant at the 10 min exposure time. At all of the concentrations tested, whole-blood AChE activity levels were depressed a minimum of 85% of control for at least 24 hr. Lastly, the VX-G Regeneration assay was successfully used as a biomarker for the presence of VX in the blood and tissues. Elevated levels of VX-G were found in the plasma at 1 hr post-exposure and in the kidney and lungs at 14 days post-exposure. There was no discernible correlation between increasing dosage of VX and the amount of VX-G found in the blood and tissue samples. Insofar as severe and potentially lethal effects of VX vapor exposure impact

operational effectiveness, the results of the current study are critical to operational risk management.

FIGURES

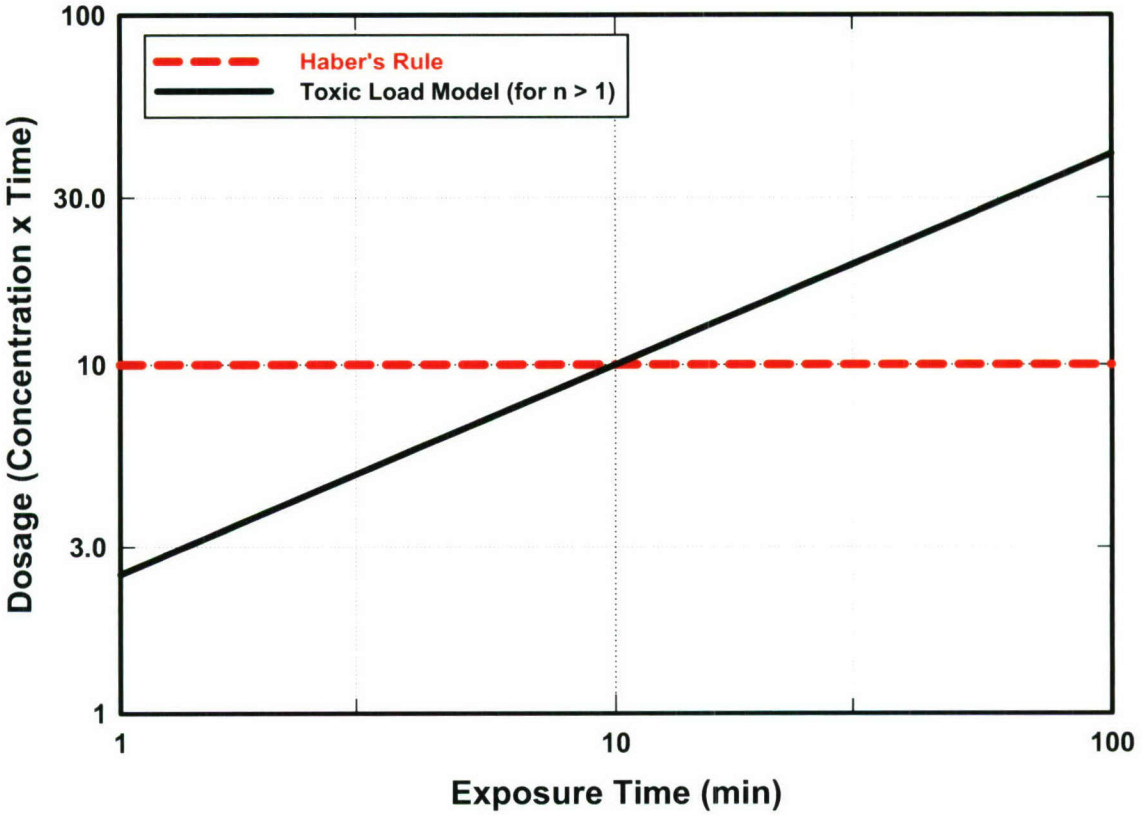
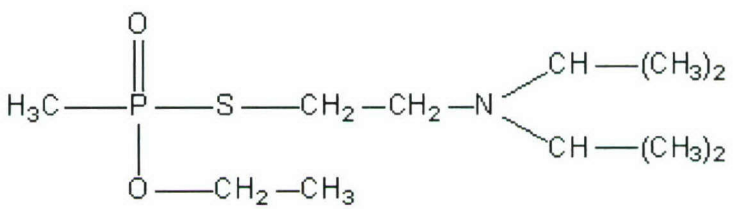


Figure 1. Comparison of Haber's Rule and Toxic Load Models for Toxicity Time Dependence



Mol. Wt. 267 g mol^{-1}

$C_{11}H_{26}NO_2PS$

(CAS Registry Numbers: 50782-69-9, 51848-47-6, 53800-40-1, 70938-84-0)

Figure 2. Structure of VX

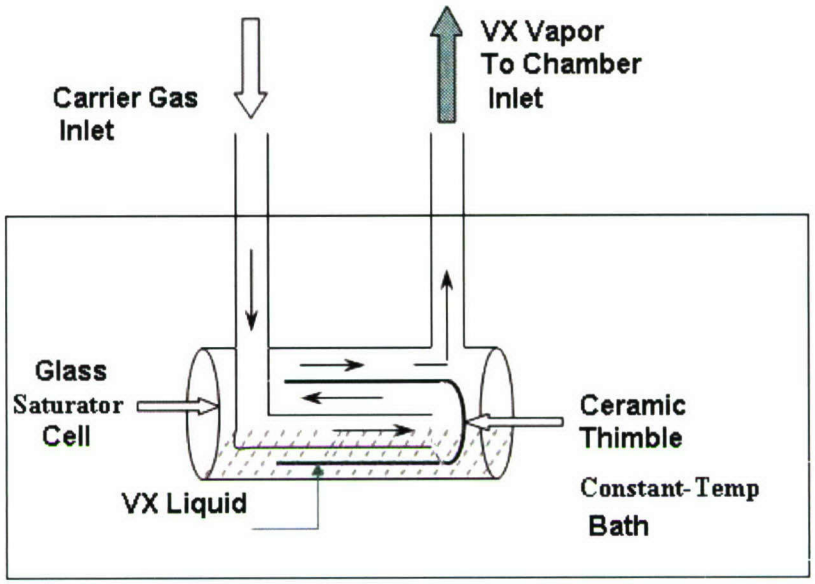


Figure 3. VX Vapor Generation Using a Saturator Cell

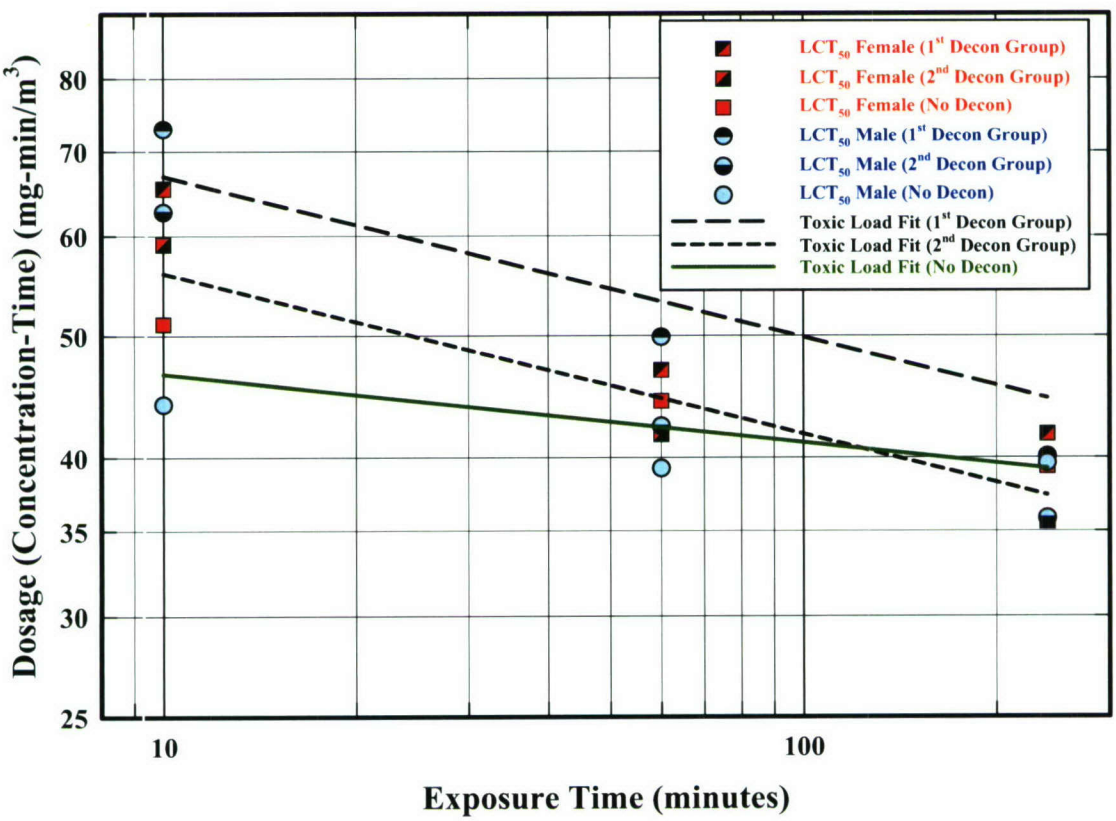


Figure 4. Comparison of Toxic Load Model Fit with VX LCT_{50} Estimates for Male and Female Rats as a Function of Exposure Duration and Group

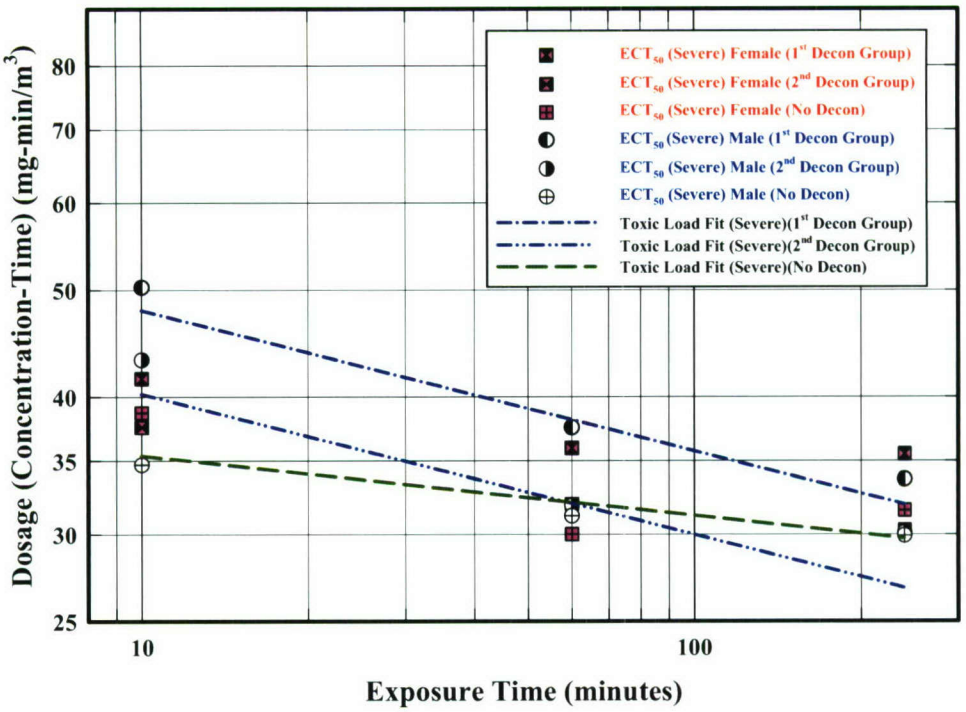


Figure 5. Comparison of Toxic Load Model Fit with VX ECT_{50} (Severe) Estimates for Male and Female Rats as a Function of Exposure Duration and Group

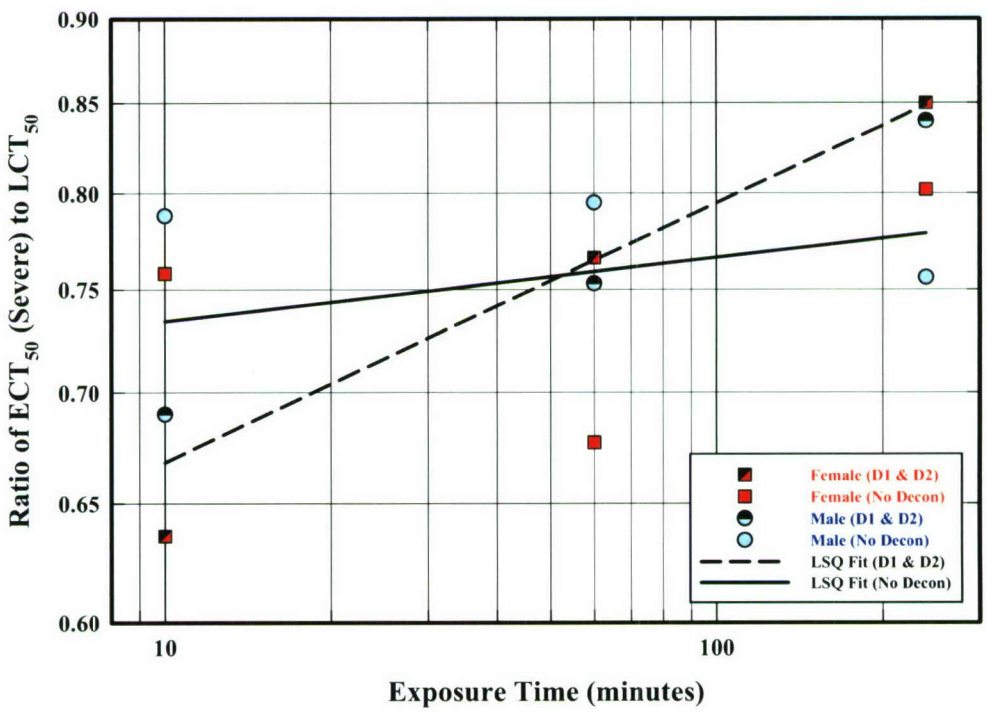


Figure 6. Ratio of Median Effective Dosages (ECT_{50} (Severe) to LCT_{50}) for Male and Female Rats as a Function of Exposure Duration and Group

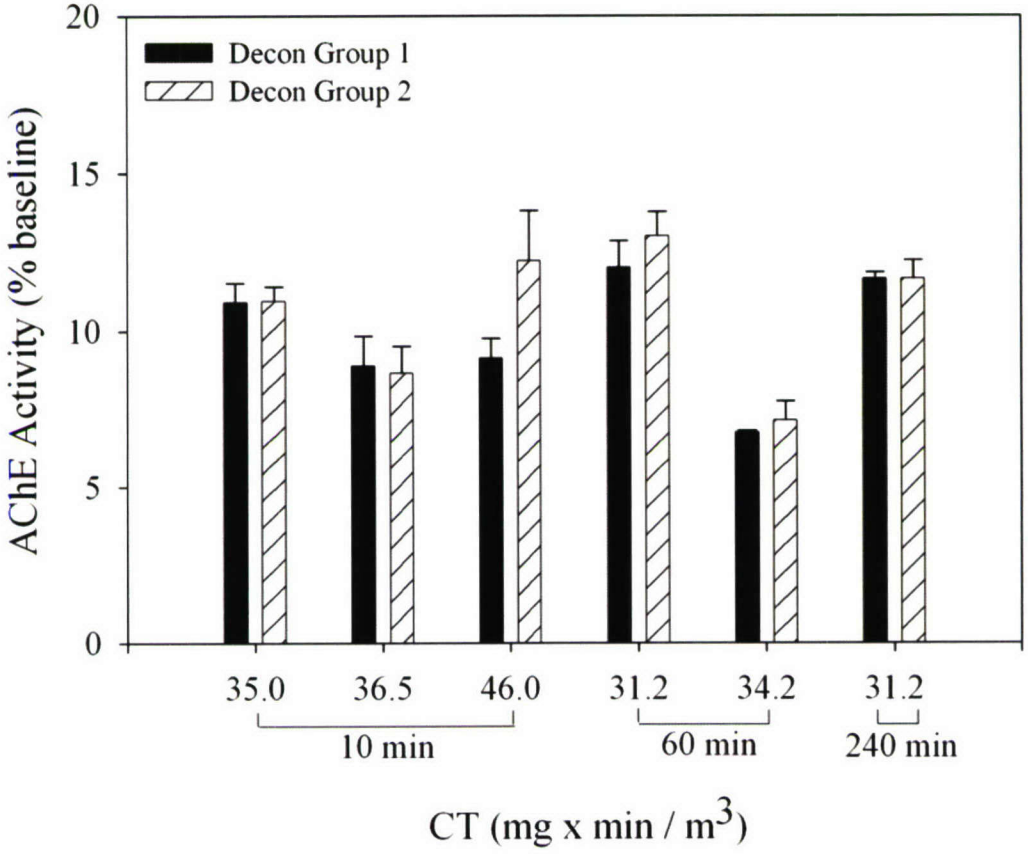


Figure 7. Effect of Decontamination Order on Whole Blood AChE Activity at 1 hr Post-Exposure, n = 3-10 for Each Bar

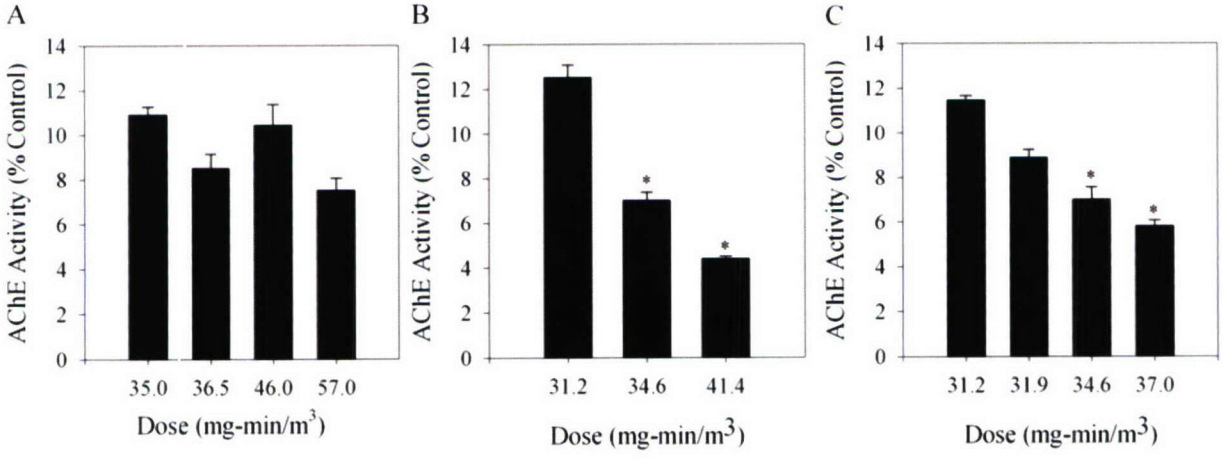


Figure 8. Effect of VX Vapor on Whole Blood AChE Activity at 1 hr Post-Exposure (A) 10 min Exposures (B) 60 min Exposures (C) 240 min Exposures: n = 4-19. * P < 0.05 relative to the lowest concentration for all exposure durations. D1 and D2 rats grouped together

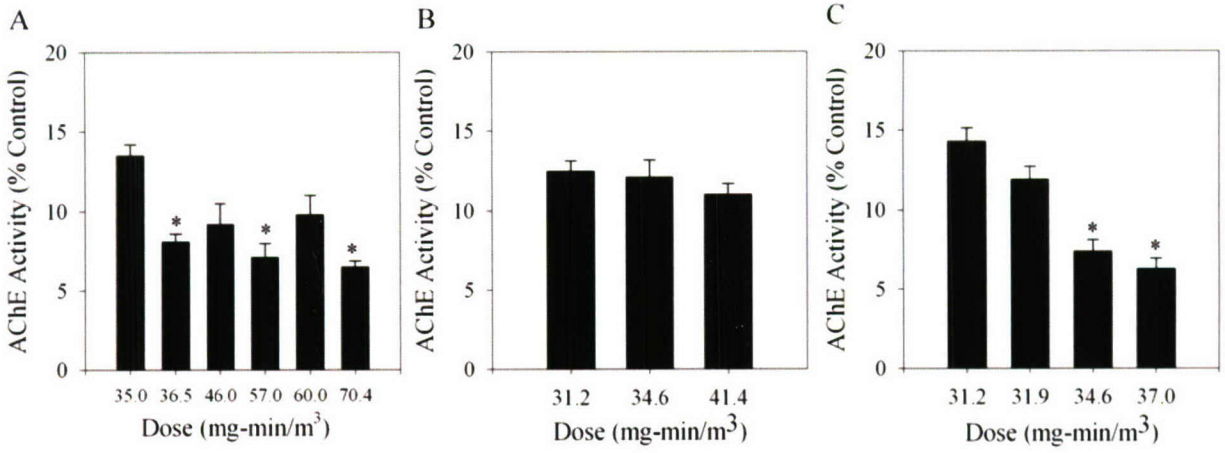


Figure 9. Effect of VX Vapor on Whole Blood AChE Activity at 24 hr Post-Exposure (A) 10 min exposures (B) 60 min exposures (C) 240 min exposures: n = 4-19. * P < 0.05 relative to the lowest concentration for all exposure durations. D1 and D2 rats grouped together

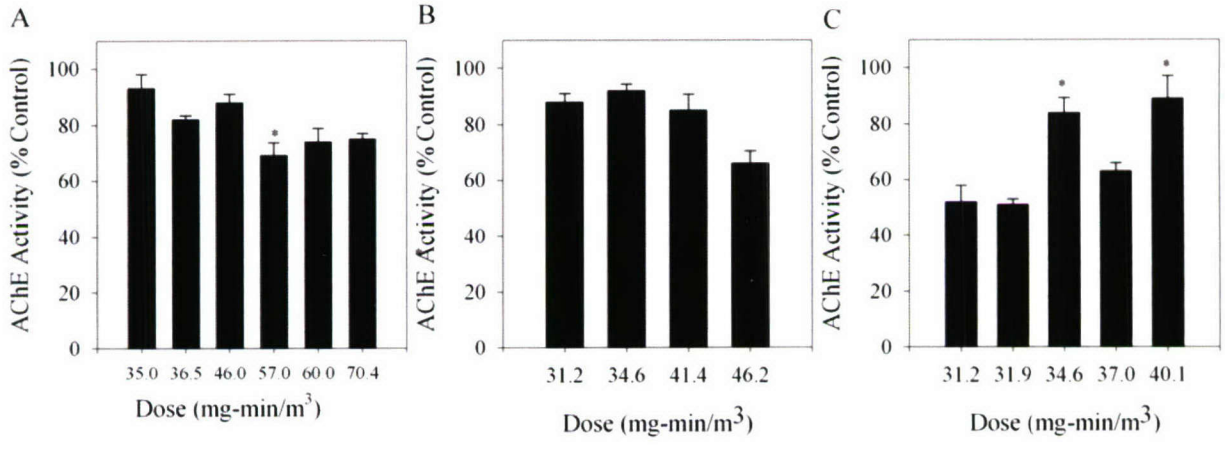


Figure 10. Effect of VX Vapor on Whole Blood AChE Activity at 7 days Post-Exposure (A) 10 min exposures (B) 60 min exposures (C) 240 min exposures: n = 4-20. * P < 0.05 relative to the lowest concentration for all exposure durations. D1 and D2 rats grouped together

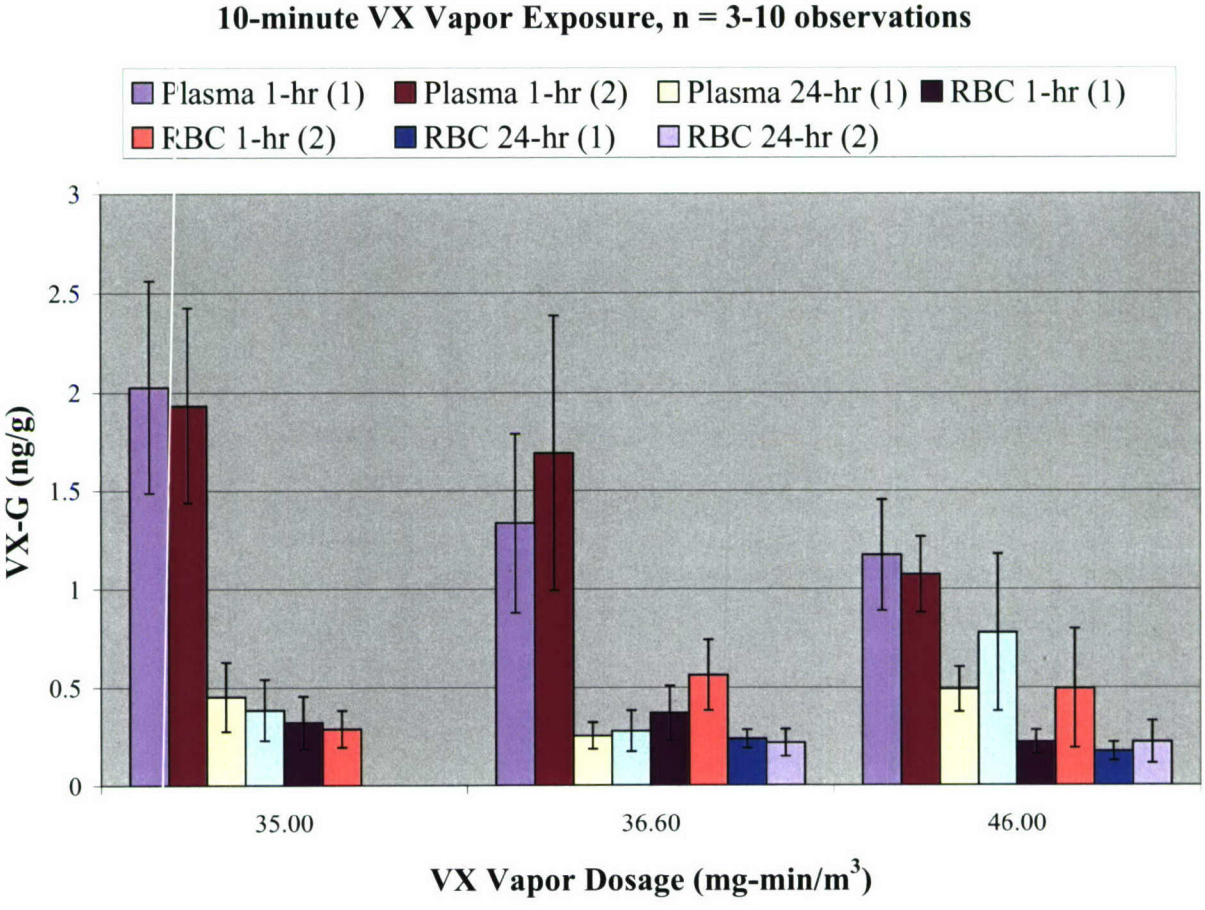


Figure 11. Effect of 10 min VX Vapor Exposures on Levels of VX-G in Plasma and RBC's at 1 hr and 24 hr Post-Exposure in Decon Groups 1 and 2

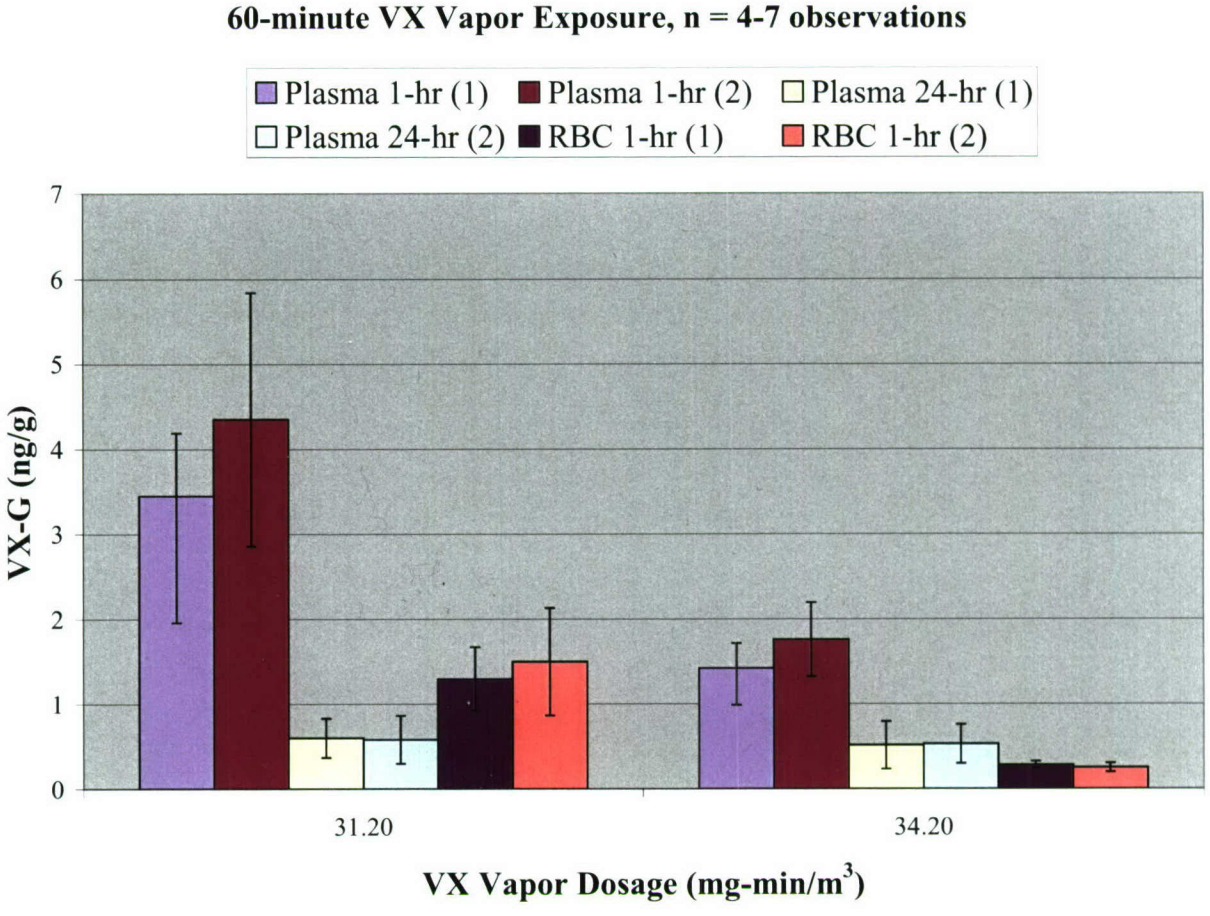


Figure 12. Effect of 60 min VX Vapor Exposures on Levels of VX-G in Plasma and RBC's at 1 hr and 24 hr Post-Exposure in Decon Groups 1 and 2

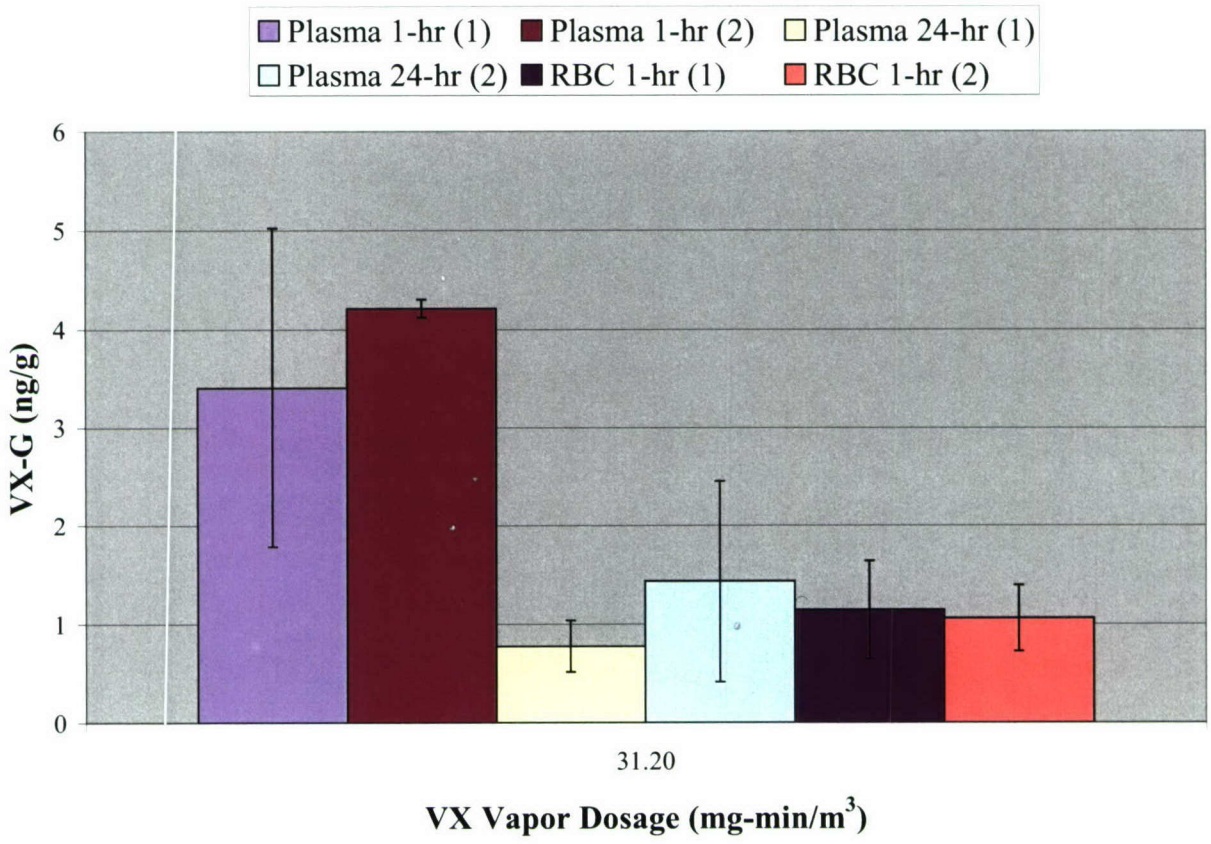


Figure 13. Effect of a 240 min VX Vapor Exposure on Levels of VX-G in Plasma and RBC's at 1 hr and 24 hr Post-Exposure in Decon Groups 1 and 2

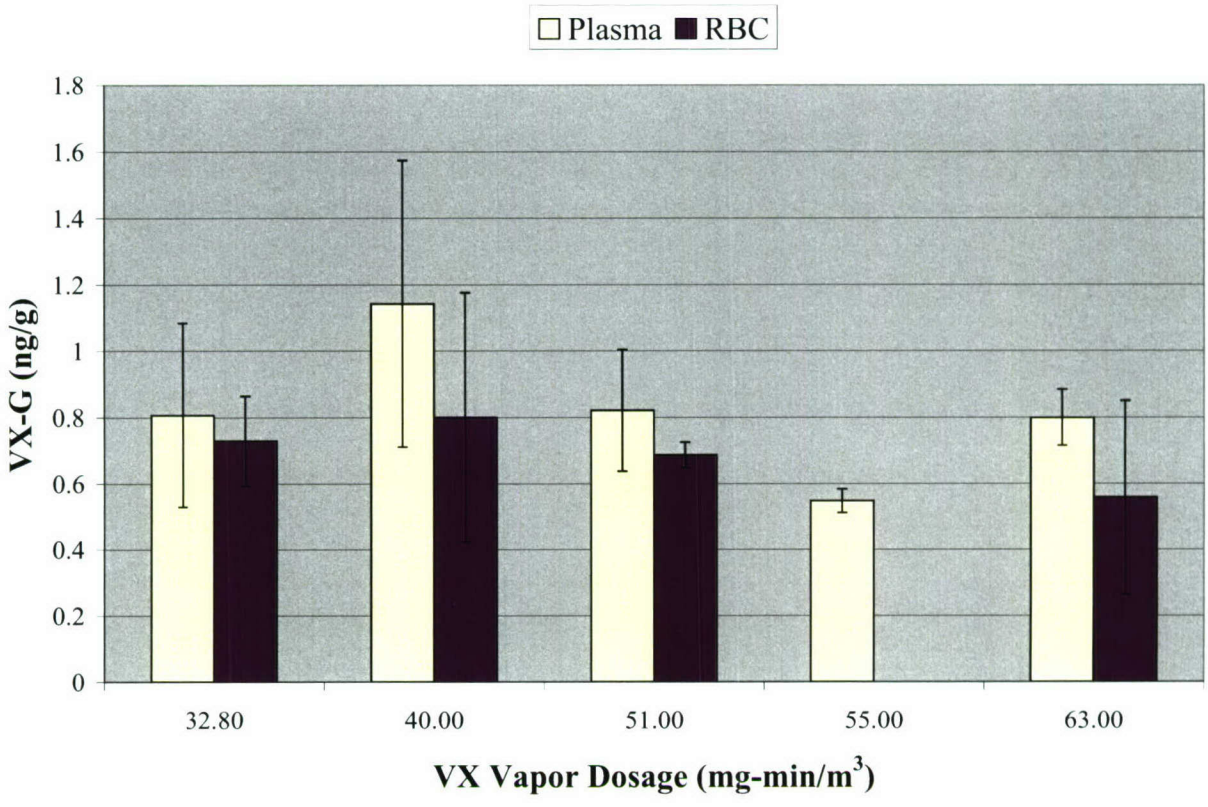


Figure 14. Effect of 10 min VX Vapor Exposures on Levels of VX-G in Plasma and RBC's at 24 hr Post-Exposure for Rats Not Decontaminated

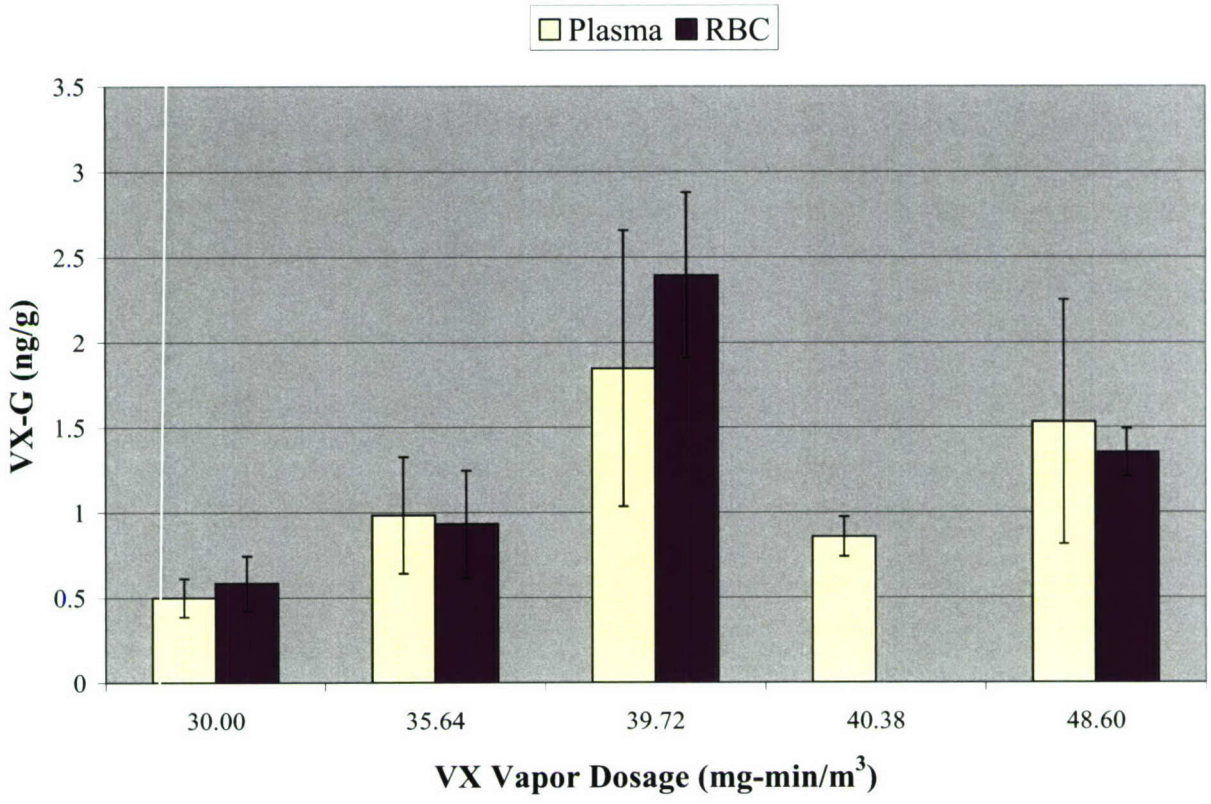


Figure 15. Effect of 60 min VX Vapor Exposures on Levels of VX-G in Plasma and RBC's at 24 hr Post-Exposure for Rats Not Decontaminated

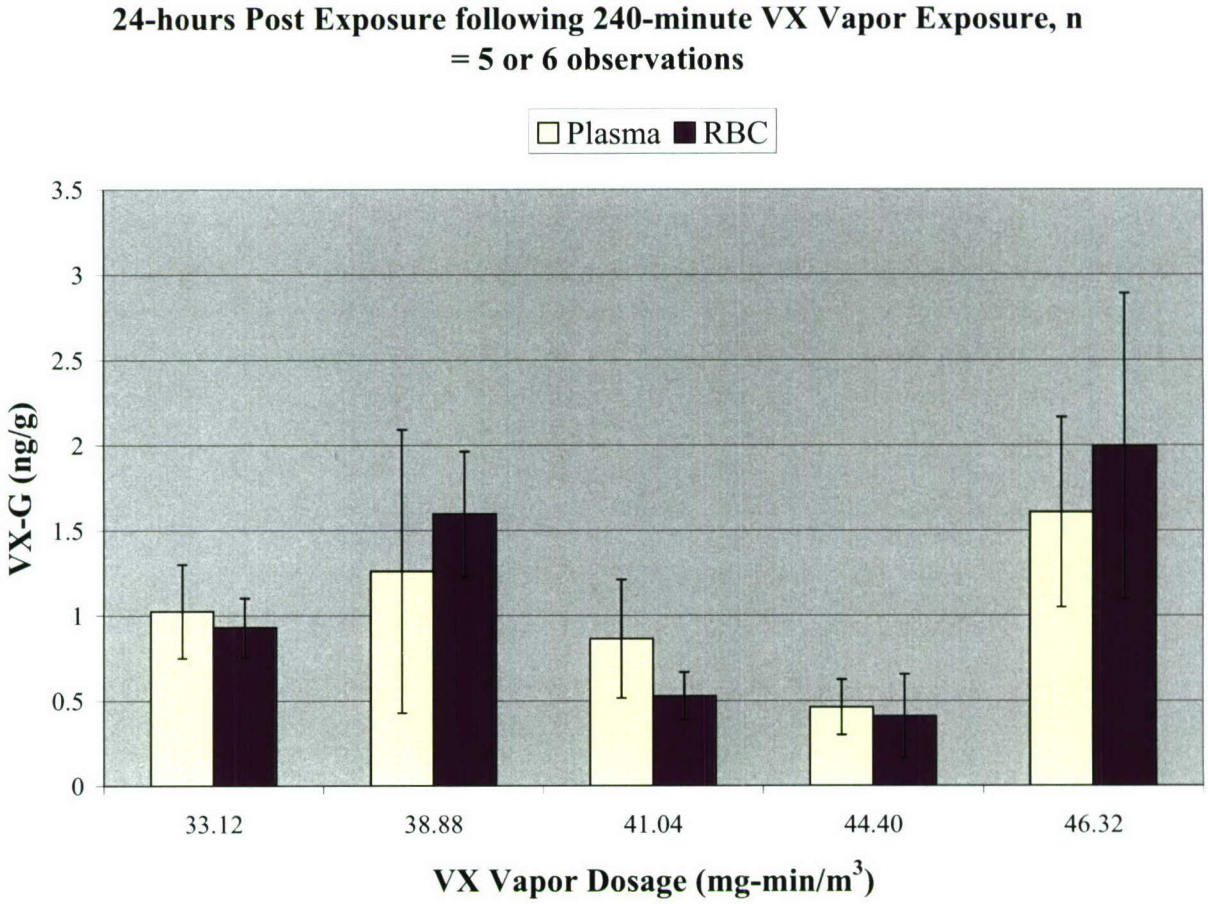


Figure 16. Effect of 240 min VX Vapor Exposures on Levels of VX-G in Plasma and RBC's at 24 hr Post-Exposure for Rats Not Decontaminated

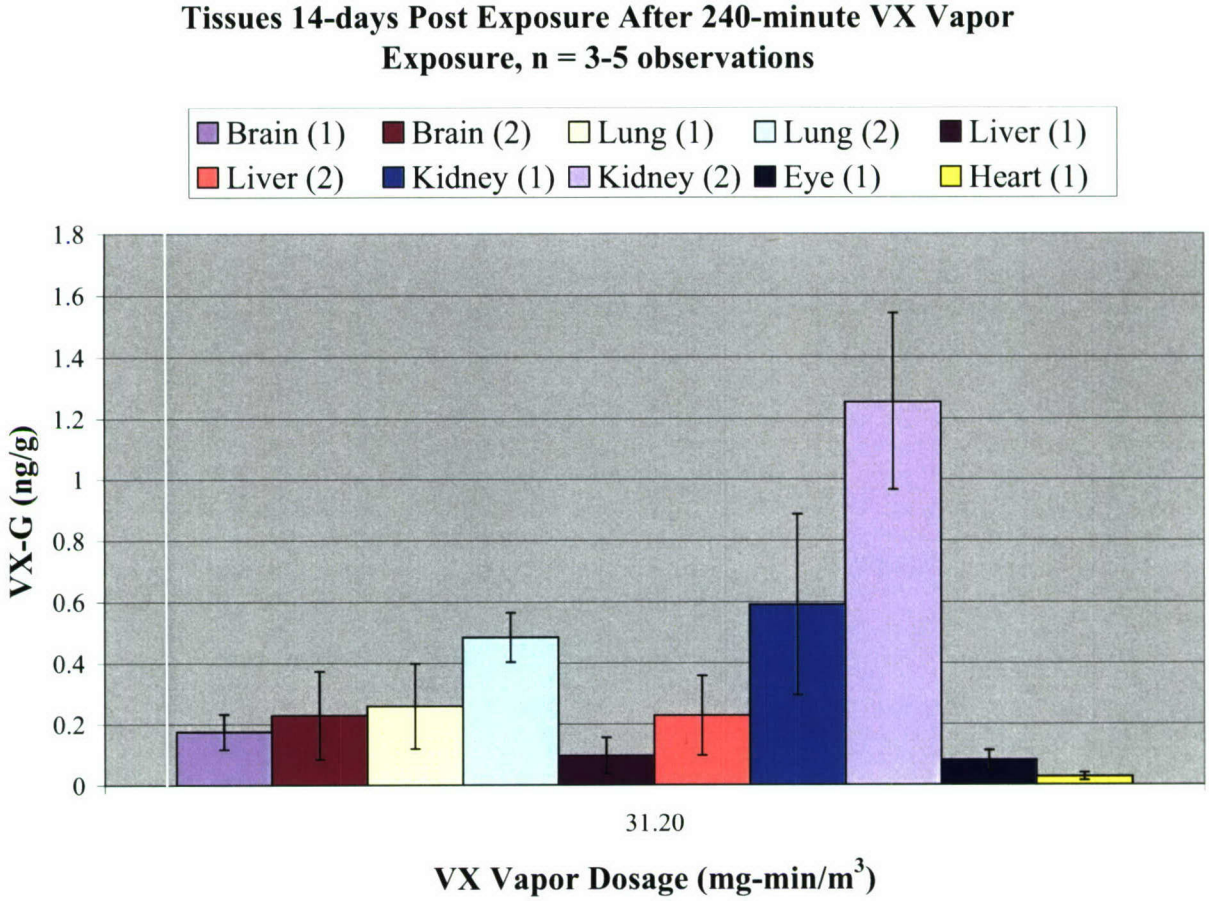


Figure 17. Distribution of VX-G in Various Tissues 14 days after a 240 min Exposure to VX-Vapor

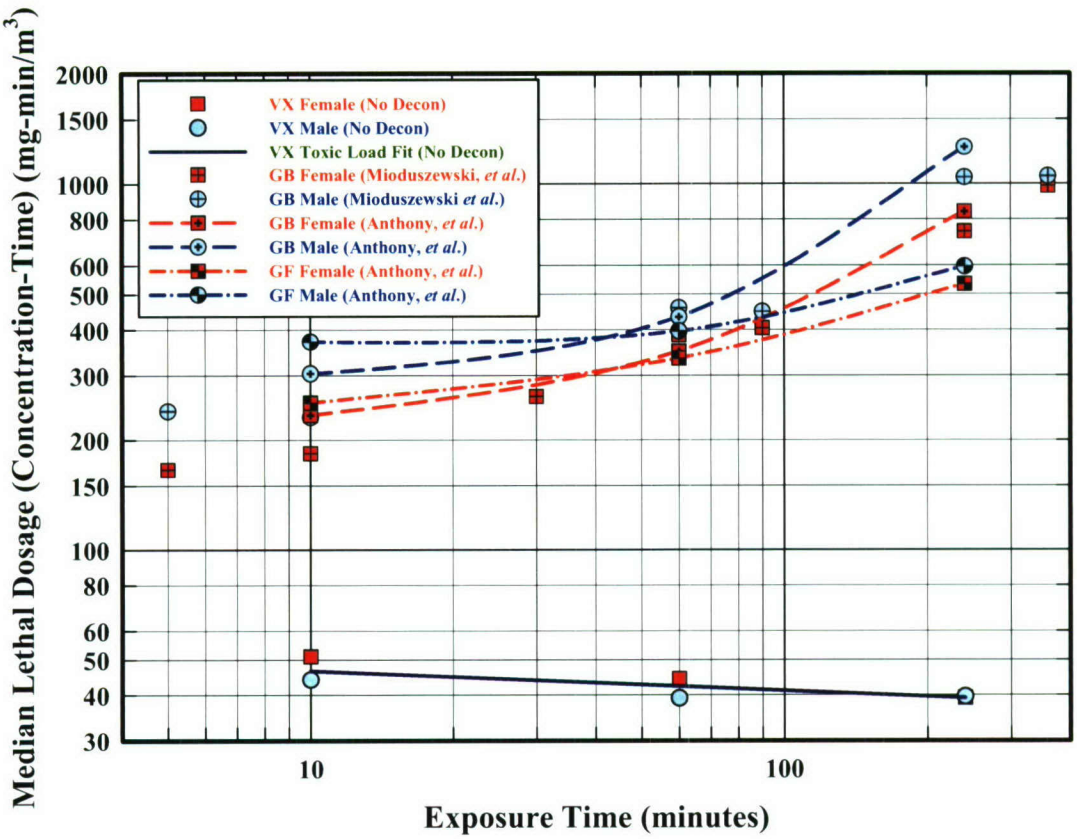


Figure 18. Comparison of Rat VX Inhalation Lethality Data with Previous Rat GB and GF Inhalation Studies

TABLES

Table 1. Definition of $Decon_j$ and Contrasts Used in Ordinal Regression Analysis of Rat VX Lethality Data

Decon Group	$Decon_1$	$Decon_2$	$Decon_3$	$\overline{D}$	$\overline{R}$
D1	1	0	0	1	1
D2	0	1	0	0	-1
ND	0	0	1	-1	0

Table 2. ECT_{50} (Severe), LCT_{50}, and 95% Fiducial Intervals for VX Vapor-induced Toxicity in Rats at 10, 60 and 240 min for Decon Groups 1 and 2 and No Decon (ND).

			Values Calculated for 24 hr Period					
			ECT_{50} (mg-min/m^3)			LCT_{50} (mg-min/m^3)		
	Time		Severe	95% Fiducial Limits		Lethality	95% Fiducial Limits	
	(min)	Group	Toxicity	lower	upper		lower	upper
		D1	41.6	37.5	46.1	65.4	59.3	72.2
	10	D2	37.5	33.6	42.0	59.1	53.8	64.8
		ND	40.9	36.9	45.2	54.4	49.8	59.5
		D1	36.0	32.6	39.6	46.9	41.6	52.9
Female Rats	60	D2	32.0	28.5	35.8	41.7	37.9	46.0
		ND	30.0	26.2	34.4	44.4	40.0	49.1
		D1	35.5	33.7	37.4	41.8	38.6	45.2
	240	D2	30.3	28.2	32.4	35.6	33.7	37.5
		ND	31.5	27.6	36.1	39.4	36.8	42.1
		D1	50.3	45.6	55.5	72.9	65.2	81.6
	10	D2	43.2	38.9	48.1	62.7	56.7	69.3
		ND	35.2	30.1	41.1	48.5	43.6	54.0
		D1	37.5	33.0	42.6	49.9	40.8	61.0
Male Rats	60	D2	31.9	26.8	37.9	42.4	36.8	48.8
		ND	31.2	28.4	34.3	39.2	36.8	41.8
		D1	33.7	31.7	35.7	40.1	37.0	43.3
	240	D2	30.1	27.8	32.6	35.8	33.7	38.0
		ND	29.9	24.5	36.6	39.6	36.4	43.1

Table 3. Probit Slopes and Standard Errors for VX Vapor-induced Toxicity in Rats at 10, 60 and 240 min for Decon Groups 1 and 2 and No Decon (ND) (24 hr Period)

		Female Rats			Male Rats		
Time (min)	Decon Group	Probit Slope	SE Slope	# of Animals	Probit Slope	SE Slope	# of Animals
10	D1	11.5	1.9	35	10.8	1.6	35
	D2			35			35
	ND	11.0	2.2	50	8.3	1.9	50
	ND w/o O	15.9	3.2	40	13.9	3.0	40
60	D1	12.4	3.0	24	8.5	2.7	26
	D2			26			24
	ND	10.5	2.7	50	14.2	3.1	50
240	D1	23.1	4.9	25	19.7	4.5	25
	D2			25			25
	ND	13.3	3.6	49	10.1	3.4	50
		Genders Combined					
		Ordinal Regression			Weighted Average		
Durations Combined	D1	10.6	0.9	170	11.8	1.0	170
	D2			170			170
	ND	10.7	1.0	299	10.6	1.1	299
	ND w/o O	12.9	1.2	279	12.9	1.3	279
	All groups	10.3	0.7	639	11.2	0.7	639
	All groups w/o O	11.2	0.7	619	12.3	0.8	619

Table 4. Ratios of ECT_{50} (Severe) to LCT_{50}, and 95% Confidence Limits for VX Vapor-induced Toxicity in Rats at 10, 60 and 240 min for Decon Groups 1 and 2 and No Decon (ND) (24 hr Period)

		Female Rats			Male Rats		
Time (min)	Group	Severe/ Lethal	95% Conf. Limits lower	95% Conf. Limits upper	Severe/ Lethal	95% Conf. Limits lower	95% Conf. Limits upper
10	D1	0.636	0.565	0.715	0.690	0.613	0.775
	D2						
	ND	0.751	0.663	0.850	0.725	0.615	0.854
	ND w/o O	0.758	0.678	0.847	0.788	0.698	0.889
60	D1	0.766	0.673	0.871	0.753	0.621	0.912
	D2						
	ND	0.677	0.560	0.818	0.795	0.715	0.885
240	D1	0.850	0.791	0.913	0.840	0.775	0.911
	D2						
	ND	0.802	0.706	0.911	0.756	0.624	0.916

		Genders Combined Ordinal Regression		
Durations Combined	D1	0.723	0.684	0.765
	D2			
	ND	0.743	0.699	0.790
	ND w/o O	0.769	0.730	0.810
	All groups	0.727	0.697	0.759
	All groups w/o O	0.739	0.711	0.769

Table 5. Effect of Decontamination Order on Whole Blood AChE Activity at 24 hr and 1 wk Post-Exposure

* P < 0.05 relative to the first decontamination group (D1)

CT (mg x min /m³)	Duration of Exposure (min)	Decon Group	AChE Activity (% baseline) 24 hr post-exp	n	AChE Activity (% baseline) 1 wk post-exp	n
35.0	10	1	14.7 ± 1.3	9	79.7 ± 7.0	6
		2	12.5 ± 0.7	10	101.3 ± 5.6	9
36.5	10	1	8.0 ± 0.8	7	82.8 ±1.4	6
		2	8.4 ± 0.8	8	83.0 ± 1.9	8
46.0	10	1	9.7 ± 2.1	5	89.0 ± 4.9	6
		2	8.4 ± 0.8	3	87.4 ± 2.8	5
57.0	10	1	8.1 ± 1.0	7	70.7 ± 6.1	9
		2	4.8 ± 0.5	3	65.4 ± 0.9	3
31.2	60	1	12.0 ± 0.8	6	87.5 ± 5.7	10
		2	13.0 ± 0.8	7	88.7 ± 2.4	10
34.2	60	1	10.2 ± 1.1	5	91.1 ± 2.7	6
		2	13.3 ± 1.6	8	93.1 ± 3.6	9
31.2	240	1	15.9 ± 0.9	10	55.9 ± 8.9	10
		2	11.1 ± 0.7*	5	46.2 ± 6.9	7
31.9	240	1	12.8 ± 0.9	7	72.0 ± 2.6	9
		2	9.8 ± 1.5	3	71.0 ± 4.1	5
34.6	240	1	7.6 ± 0.5	9	83.8 ± 5.6	9
		2	6.6 ± 0.1	3	85.7 ± 15.8	3

Table 6. Summary of CW Nerve Agent Inhalation Studies Involving Rats Conducted Under the Low Level Toxicology Program

Name of Study	Mioduszewski, *et al*. (2002a,b)	Anthony, *et al*. (2004)	Present Study
Subsequent Analysis	Sommerville (2004)		
Agent(s) Investigated	GB	GB and GF	VX
Year(s) Conducted	1998 to 2000 in two phases	2001 to 2002	2005 in two phases
Total Number of Subjects	700	500	640
Gender	Equal Number Males and Females	Males (240) Females (260)	Equal Number Males and Females
Breakdown by Agent of Number of Subjects	All GB	GF (320); GB (180)	All VX
Breakdown by Special Handling of Subjects	No special handling	No special handling	Decon Post-Exposure (340); No Decon (300)
Number of Subjects per Exposure Group	10 or 20	5, 10 or 20	20
Number of Runs	43	38	32
Vapor Concentrations (mg/m3)	GB: 2.0 to 54.4	GB: 3.5 to 35.9	VX: 0.138 to 7.05
		GF: 2.0 to 41.9	
Exposure Times (minutes)	Phase I: 10, 30, 90, 240 Phase II: 5, 60, 360	10, 60 and 240	10, 60 and 240
Primary Endpoint(s) of Interest	Lethality (1 and 14 days)	Lethality (1 and 14 days)	Lethality and Severe Effects (1 and 14 days)
Toxic Load Exponent (n)	GB: 1.66	GB: 1.71	VX: 0.92 (no decon)
		GF: 1.24	
Presence of Curvature with Respect to Exposure Duration	yes	yes	no
Overall Probit Slope	13.9	18 for both agents	10.4 (no difference between decon and no decon)
Ratio of Median Effective Dosages (Severe to Lethal)	0.79	0.83 for both agents	0.75 (no decon)
Gender Differences	Female more sensitive with difference greatest at shorter durations	Female more sensitive with difference greatest at shorter durations	Male more sensitive only at the shortest duration (10 minutes)

Blank

LITERATURE CITED

Agresti, A. Categorical Data Analysis. John Wiley & Sons, New York (1990).

Anthony, J.S., Haley, M., Manthei, J., Way, R., Burnett, D., Gaviola, B., Sommerville, D., Crosier, R., Mioduszewski, R., Thomson, S., Crouse, C., Matson, K. "Inhalation Toxicity of Cyclosarin (GF) Vapor in Rats as a Function of Exposure Concentration and Duration: Potency Comparison to Sarin (GB)," Inhalation Toxicology, 16: 103-111, 2004.

Bide, R.W. and Risk, D.J. "Inhalation Toxicity of Aerosolized Nerve Agents. 1. VX Revisited. Defence Research Establishment Suffield." Technical Report DRES TR 2000-063, September 2000 (Unclassified Report).

Bide, R.W., Risk, D.J., Beifus, D.G. "A Functional Small Volume Inhalation System for Highly Toxic Materials." Proceedings of the 1996 ERDEC Scientific Conference on Chemical and Biological Defense Research, 19-22 November 1996, AD-A334 105, pages 25-32.

Booth, J. and Gillette, J.R. "The effect of anabolic steroids on drug metabolism by microsomal enzymes in rat liver." J. Pharmacol. Exp. Ther. sep;137:374-9 (1962).

Bours, J., Fink, H., and Hockwin, O. "The quantification of eight enzymes from the ageing rat lens, with respect to sex differences and special reference to aldolase." Curr. Eye Res. May 7(5):449-55 (1988)

Brickhouse, M.D., Rees, M.S., O'Connor, R.J., Durst, H.D. "Nuclear Magnetic Resonance (NMR) Analysis of G-class and VX Nerve Agents and Reaction Masses Produced by Their Chemical Neutralization," ERDEC-TR-449; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1997; UNCLASSIFIED Report (AD-A339 308).

Craig, F.N., Cummings, E.G. and Sim, V.M. "Environmental temperature and the percutaneous absorption of a cholinesterase inhibitor, VX," J Invest Dermatol 68(6), 357-361 (1977).

Crook, J.W., Hott, P., Owens, E.J., Cummings, E.G., Farrand, R.L., Cooper, A.E. The Effects of Subacute Exposures of the Mouse, Rat, Guinea Pig, and Rabbit to Low-Level VX Concentrations. ARCSL-TR-82038, June 1983 (Unclassified).

Dimmick, R.L. Jr., Owens, E.J., Farrand, R.L. "The Acute Inhalation Toxicity of QL in the Rat and Guinea Pig." ARCSL-TR-79031, 1979, (Unclassified).

Edgewood Chemical Biological Center (ECBC), "Materials Safety Data Sheet for Lethal Nerve Agent (VX)," US Army RDECOM, Aberdeen Proving Ground, MD, Revised 10 August 2004.

Ellman, G.L., Courtney, K.D., Andres, V., Jr., and Featherstone, R.M. "A new rapid colormetric determination of acetylcholinesterase activity." Biochem. Pharm. 7:88-95 (1961).

Fairhurst, S. and Turner R.M. "Toxicological Assessments in Relation to Major Hazards." Journal of Hazardous Materials. 33: 215-227 (1993).

Finney, D.J. Probit Analysis. 3rd Edition, Cambridge University Press, Cambridge (1971).

Fox, J. Applied Regression Analysis, Linear Models, and Related Methods. SAGE Publications, Inc., Thousand Oaks, CA (1997).

Griffiths, R.F. "The Use of Probit Expressions in the Assessment of Acute Population Impact of Toxic Releases." J. Loss Prev. Process Ind., 4: 49-57 (1991).

Gupta, R.C., Patterson, G.T. and Dettbarn, W.D. "Comparison of Cholinergic and Neuromuscular Toxicity following Acute Exposure to Sarin and VX in Rat." Fundamental and Applied Toxicology 16(3), 449-458 (1991).

Haber, F.R. Zur geschichte des gaskrieges. In "Funf Vortrage aus Jahren 1920-1923", Spinger, Berlin, 1924.

Hartmann, H.M. "Evaluation of Risk Assessment Guideline Levels for the Chemical Warfare Agents Mustard, GB, and VX." Regulatory Toxicology and Pharmacology 35, 347-356 (2002).

Holck, H.G.O., Kanan, M.A., Mills, L.M. and Smith, E.L. "Studies Upon the Sex-Difference in Rats in Tolerance to Certain Barbiturates and to Nicotine." J. Pharmacol. Exp. Ther., 60, 323 (1937).

Hulet, S.W., Sommerville, D.R., Benton, B.J., Forster, J.S., Manthei, J.H., Miller, D.B., Scotto, J.A., Jarvis, J.R., Way, R.A., Muse, W.T., Gaviola, B., Burnett, D., Crosier, R.B., Mioduszewski, R.J., and Thomson, S.A. "Low-Level Sarin Vapor Exposure in the Gottingen Minipig: Effect of Exposure Concentration and Duration on Pupil Size." Edgewood Chemical Biological Center, APG, MD. ECBC-TR-450, 2006 (in preparation).

Jakubowski, E.M., Heykamp, L. S., Durst, H.D., and Thomson, S.A. "Preliminary Studies in the Formation of Ethyl Methylphosphonofluoridate from Rat and Human Serum Exposed to VX and Treated with Fluoride Ion." Anal. Letters, 34(5): 727-737 (2001).

Jakubowski, E.M., Benton, B.J., Whalley, C.E., Anthony, J.S., Haley, M.V., Manthei, J.H., Way, R.A., Burnett, D.C., Gaviola, B.P., Scotto, J.A., Sommerville, D.R., Crosier, R.B., Edwards, J.L., Evans, R.A., McGuire, J.M., Crouse, C.L., Matson, K.L., Mioduszewski, R.J. and Thomson, S.A. "The Inhalation Toxicity Testing of VX Vapor in Rats at Miosis Levels: VX Surface Contamination Analysis and Fluoride Ion Generated Product Determination." Edgewood Chemical Biological Center, APG, MD. ECBC-TR-XXX, 2006 (in preparation).

Kato, R. "Sex-Related Differences in Drug Metabolism." Drug Metabolism Reviews, 3(1), 1-32 (1974).

Kato, R., Chiesara, E., Vassanelli, P. "Metabolic differences of strychnine in the rat in relation to sex." Jpn J Pharmacol. Jun;12:26-33 (1962a).

Kato, R., Chiesara, E., Vassanelli, P. "Increased activity of microsomal strychnine-metabolizing enzyme induced by phenobarbital and other drugs." Biochem Pharmacol. Oct;11:913-22 (1962b).

Lundy, P.M., Hamilton, M.G., Hill, I., Conley, J., Sawyer, T.W., Caneva, D.C. "Clinical Aspects of Percutaneous Poisoning by the Chemical Warfare Agent VX: Effects of Application Site and Decontamination." Military Medicine, 169, 11:856, 2004.

Manthei, J.H., Beckett, E.L., Heitkamp, D.H., James, J.T., Cameron, K.P., Bona, D.M., Moore, R.D., Vickers, E.L. "Toxic Hazard Evaluation of Decontaminated/Neutralized Chemical Surety Materials Waste at the U.S. Army Chemical Research, Development and Engineering Center." US Army CRDEC, APG, MD. CRDEC-TR-146, November 1990, (Unclassified).

Martin, B.R. "Biodisposition of Organophosphates in Guinea Pigs and Mice after Intramuscular, Intravenous and Inhalation Exposure." AD-A247 762, June 1991, (Unclassified).

Maxwell, D.M. "The Specificity of Carboxylesterase Protection against the Toxicity of Organophosphorus Compounds." Toxicology and Applied Pharmacology 114, 306-312 (1992).

McNamara, B.P., Vocci, F.J., Leitnaker, F.C. Proposed Limits for Human Exposure to VX Vapor in Nonmilitary Operations. EASP 1100-1 (R-1), July 1973, (Unclassified).

Miller, D.B. Benton, B.J., Hulet, S.W., Mioduszewski, R.J., Whalley, C.E., Carpin, J.C., and Thomson, S.A. "An automated infrared image acquisition and analysis method for quantifying optical responses to chemical agent vapor exposure." 29th Annual IEEE Northeast Bioengineering Conference, 22-23 March 2003, New Jersey Institute of Technology, Newark, NJ, 2003a.

Miller, D.B. Benton, B.J., Hulet, S.W., Mioduszewski, R.J., Whalley, C.E., Carpin, J.C., and Thomson, S.A. "An image analysis method for quantifying elliptical and partially obstructed pupil areas in response to chemical agent vapor exposure." 29th Annual IEEE Northeast Bioengineering Conference, 22-23 March 2003, New Jersey Institute of Technology, Newark, NJ, 2003b.

Miller, D.B., Benton, B.J., Hulet, S. W., Mioduszewski, R.J., Whalley, C.E., Carpin, J.C., and Thomson, S.A. "An infrared image acquisition and analysis method for quantifying optical responses to chemical agent vapor exposure." 2002 Joint Services Scientific Conference on Chemical & Biological Defense, 19-21 November 2002, Hunt Valley Maryland.

Mioduszewski, R.J., Manthei, J.H., Way, R.A., Burnett, D.C., Gaviola, B.I., Muse, W.T. Jr., Matson , K.L., Sommerville, D.R., Crosier, R.B., Scotto, J.A., McCaskey, D.A., Crouse, C.L. and Thomson, S.A. "Low-Level Sarin Vapor Exposure in Rats: Effect of Exposure Concentration and Duration on Pupil Size." ECBC-TR-235, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, May 2002a (Unclassified Report).

Mioduszewski, R.J., Manthei, J.H., Way, R.A., Burnett, D.C., Gaviola, B.P., Muse, W.T., Thomson, S.A., Sommerville, D.R., and Crosier, R.B., "Interaction of exposure concentration and duration in determining acute toxic effects of sarin vapor in rats." Toxicol. Sci. 66: 176-184 (2002b).

Muse, W.T. Jr., Anthony, J.S., Tomson, S.A., Crouse, C.L., Crouse, L.C.B. "Acute Inhalation Toxicity of Chemically Neutralized/Hydrolyzed VX in Rats. SCWO Effluent Prior to Evaporation SCWO Effluent Post-Evaporation." ECBC-TR-219, April 2002, (Unclassified Report).

Oshiro, I., Takenaka, T., Maeda, J. "New Method for Hemoglobin Determination by Using Sodium Lauryl Sulfate (SLS)." Clin. Biochem. 15(1) 83-88 (1982).

Prins, J, "Product and Process Comparison," Chapter 7 in Croarkin, C, and Tobias, P, eds., NIST/SEMATECH e-Handbook of Statistical Methods. http://www.itl.nist.gov/div898/handbook/. Last revised November 2003, National Institute of Standards and Technology, Washington, DC, 8 July 2004.

2000 Report of the AVMA Panel on Euthanasia. JAVMA, Vol. 218, March 1, 2001.

Rickett, D.L., Glenn, J.F. and Beers, E.T. "Central Respiratory Effects VX. Neuromuscular Actions of Nerve Agents." Neurotoxicology 7(1), 225-236 (1986).

Silver, A. The Biology of Cholinesterases: Frontiers of Biology 36, Elsevier/North-Holland, Amsterdam (1974).

Sommerville, D.R., "Relationship between the Dose Response Curves for Lethality and Severe Effects for Chemical Warfare Nerve Agents," Proceedings of the 2003 Joint Service

Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, ECBC-SP-018, October 2005.

Sommerville, D.R., Park, K.H., Kierzewski, M.O., Dunkel, M.D., Hutton, M.I. and Pinto, N.A., "Toxic Load Modeling," Chapter 8, pp. 137-158 in Inhalation Toxicology, Second Edition, eds. Salem, H and Katz, S.A., Taylor and Francis, Baton Raton, FL, (2006).

Sullivan, D.A., Hann, L.E., Yee, L., and Allansmith, M.R. "Age- and gender-related influence on the lacrimal gland and tears." Acta Ophthalmol (Copenh) Apr 68(2):188-94 (1990)

ten Berge, W.F., Zwart, A., and Appelman, L.M., "Concentration-time mortality response relationship of irritant and systemically acting vapours and gases". Journal of Hazardous Materials 13:301-309 (1986).

Weimer, J.T. and Ballard, T.A. "Note on the Influence of Inhaled SNA on the Inhalation Toxicity of VX to Rats," US Army Chemical Warfare Laboratories, CWL-TM-24-36, April 1960, (Unclassified).

Whalley, C.E., Benton, B.J., Manthei, J.H., Way, R.A., Jakubowski, E.M., Jr., Burnett, D.C., Gaviola, B.I., Crosier, R.B., Sommerville, D.R., Muse, W.T., Forster, J.S., Mioduszewski, R.J., Thomson, S.A., Scotto, J.A., Miller, D.B., Crouse, C.L., Matson, K.L., and Edwards, J.L. "Low-Level Cycolosarin (GF) Vapor Exposure in Rats: Effect of Exposure Concentration and Duration on Pupil Size." US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. ECBC-TR-407, September 2004, (Unclassified Report).

Whalley, C.E., Lumley, L.A., McGuire, J.M., Jakubowski, E.M., Miller, D.B., McGonough, J.H., Mioduszewski, R.J., Thomson, S.A., and Shih, T-M.A. "Toxicokinetics of Inhaled and Parenteral Sarin (GB) Following Multiple Sub-Lethal Exposures in the Guinea Pig." Proceedings of the 2005 Joint Service Scientific Conference on Chemical & Biological Defense Research, Timonium, MD, p. 108, 14-16 November 2005.

APPENDIX: ORDINAL LOGISTIC REGRESSION PRINTOUTS FROM MINITAB®

6 INTRODUCTION

Ordinal logistic regression (with a normit link function) was used to fit the ordinal responses observed in this study. The appropriate routine within MINITAB® (Version 14) was used to perform the calculations. The printouts for these analyses are included in this appendix. Comments by the analyst about the printouts are preceded by [DRS].

6.1 Nomenclature

Conc	Concentration of VX vapor in mg/m^3.
Const(x)	Fitted coefficients provided by MINITAB®, specific to level of effect. The highest x value corresponds to the greatest effect (ie. lethality).
Dcntrst	Decontamination contrast Equals 1 for decon group D1 from Part I Equals 0 for decon group D2 from Part I Equals -1 for decon group ND from Part II
Dgroup	Decontamination group Equals 1 for decon group D1 from Part I Equals -1 for decon group D2 from Part I Equals 0 for decon group ND from Part II
Dgrp	Three level factor for Decontamination group (values of D1, D2 or ND)
Gender	Male (Gender = 1) or female (Gender = -1)
GTgroup	Gender-time combination F10—Female rats exposed for 10 minutes F60—Female rats exposed for 60 minutes F240—Female rats exposed for 240 minutes M10—Male rats exposed for 10 minutes M60—Male rats exposed for 60 minutes M240—Male rats exposed for 240 minutes
Igroup	Gender-time-Dgroup combination First character—M for male and F for female Second group--Number for duration (ex. 10 for 10 minutes) Last group—D1 for decon group D1 and D2 for decon group D2 Example: F10D1 stands for 10 minute females in decon group D1
logC	Log base 10 of vapor concentration
logCT	Log base 10 of vapor concentration multiplied by exposure duration
logT	Log base 10 of exposure duration
n	Toxic load exponent
Rcntrst	Removal period contrast Equals 1 for decon group D1 from Part I Equals -1 for decon group D2 from Part I Equals 0 for decon group ND from Part II
Score[1d]	Observed level of effects within period of one day (post-exposure) Equals 1 for mild effects—S(1)

Equals 2 for moderate effects—S(2)
Equals 3 for severe effects—S(3)
Equals 4 for lethality—S(4)

SE Standard error of coefficient

T Exposure duration (in minutes)

Z Normit (Z = 0 for 50% response, -1 for 16% response and 1 for 84% response)

6.2 Summary of Ordinal Response Data from Parts I and II

6.2.1 Tabulated Statistics: Conc, Score[1d], GTgroup, and Dgroup for Part I

The number of rats exhibiting a Score[1d] value per vapor concentration, GTgroup and Dgroup is tabulated below.

Results for GTgroup = F10, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
3.480	0	1	3	0	4
3.650	4	1	0	0	5
4.640	0	0	5	1	6
5.730	0	0	1	4	5
6.040	0	0	3	2	5
6.800	0	0	1	4	5
7.050	0	0	2	3	5
All	4	2	15	14	35

Results for GTgroup = M10, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
3.480	0	4	2	0	6
3.650	4	1	0	0	5
4.640	0	2	1	1	4
5.730	0	0	2	3	5
6.040	0	0	4	1	5
6.800	0	0	1	4	5
7.050	0	0	3	2	5
All	4	7	13	11	35

Results for GTgroup = F10, Dgroup = 1

	Score[1d]				
Conc	1	2	3	4	All
3.480	1	4	1	0	6
3.650	4	1	0	0	5
4.640	0	1	2	1	4
5.730	0	0	5	0	5
6.040	0	0	3	2	5
6.800	0	0	1	4	5
7.050	0	0	3	2	5
All	5	6	15	9	35

Results for GTgroup = M10, Dgroup = 1

	Score[1d]				
Conc	1	2	3	4	All
3.480	1	2	1	0	4
3.650	5	0	0	0	5
4.640	0	4	2	0	6
5.730	0	4	1	0	5
6.040	0	0	4	1	5
6.800	0	0	2	3	5
7.050	0	0	3	2	5
All	6	10	13	6	35

Results for GTgroup = F60, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
0.523	2	2	2	0	6
0.571	0	2	2	1	5
0.667	0	0	2	3	5
0.691	0	0	3	2	5
0.775	0	0	1	4	5
All	2	4	10	10	26

Results for GTgroup = M60, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
0.523	0	2	2	0	4
0.571	0	2	3	0	5
0.667	0	1	2	2	5
0.691	0	1	2	2	5
0.775	0	0	1	4	5
All	0	6	10	8	24

Results for GTgroup = F240, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
0.130	1	2	2	0	5
0.133	0	0	4	1	5
0.144	0	0	1	4	5
0.156	0	0	3	2	5
0.167	0	0	1	4	5
All	1	2	11	11	25

Results for GTgroup = M240, Dgroup = -1

	Score[1d]				
Conc	1	2	3	4	All
0.130	0	1	4	0	5
0.133	0	0	4	1	5
0.144	0	1	2	2	5
0.156	0	1	3	1	5
0.167	0	0	0	5	5
All	0	3	13	9	25

Results for GTgroup = F60, Dgroup = 1

	Score[1d]				
Conc	1	2	3	4	All
0.523	1	2	1	0	4
0.571	0	2	1	2	5
0.667	0	2	3	0	5
0.691	0	2	3	0	5
0.775	0	0	3	2	5
All	1	8	11	4	24

Results for GTgroup = M60, Dgroup = 1

	Score[1d]				
Conc	1	2	3	4	All
0.523	2	2	2	0	6
0.571	0	2	2	1	5
0.667	0	2	1	2	5
0.691	1	3	0	1	5
0.775	0	1	2	2	5
All	3	10	7	6	26

Results for GTgroup = F240, Dgroup = 1

	Score[1d]

Conc	1	2	3	4	All
0.130	4	1	0	0	5
0.133	0	3	2	0	5
0.144	0	4	1	0	5
0.156	0	3	2	0	5
0.167	0	0	2	3	5
All	4	11	7	3	25

Results for GTgroup = M240, Dgroup = 1

	Score[1d]				
Conc	1	2	3	4	All
0.130	5	0	0	0	5
0.133	0	1	4	0	5
0.144	1	2	1	1	5
0.156	0	2	2	1	5
0.167	0	0	1	4	5
All	6	5	8	6	25

6.2.2 Tabulated Statistics: Conc, Score[1d], and GTgroup for Part II

The number of rats exhibiting a Score[1d] value per vapor concentration and GTgroup is tabulated below. One female rat escaped from her cage into the exposure chamber during a 240 minute run and was not counted in the final tally or statistical analysis.

Results for GTgroup = F10, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
3.280	10	0	0	10
4.130	2	7	1	10
5.180	0	3	7	10
5.530	3	5	2	10
6.350	0	2	8	10
All	15	17	18	50

Results for GTgroup = F60, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
0.503	5	4	1	10
0.594	2	7	1	10
0.665	0	8	2	10
0.673	2	4	4	10
0.811	0	3	7	10
All	9	26	15	50

Results for GTgroup = F240, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
0.138	2	7	1	10
0.162	2	5	3	10
0.172	2	1	7	10
0.184	0	2	8	10
0.195	0	1	8	9
All	6	16	27	49

Results for GTgroup = M10, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
3.280	8	2	0	10
4.130	0	5	5	10
5.180	0	1	9	10
5.530	2	6	2	10
6.350	0	1	9	10
All	10	15	25	50

Results for GTgroup = M60, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
0.503	5	5	0	10
0.594	1	6	3	10
0.665	1	4	5	10
0.673	2	3	5	10
0.811	0	0	10	10
All	9	18	23	50

Results for GTgroup = M240, Dgroup = 0

	Score[1d]			
Conc	2	3	4	All
0.138	3	5	2	10
0.162	0	5	5	10
0.172	3	1	6	10
0.184	0	5	5	10
0.195	0	1	9	10
All	6	17	27	50

6.3 Summary of Exposure Runs for Rat VX Lethality Study from Parts I and II

Each run involved the exposure of 20 rats (10 males and 10 females). In Part I, the rats were further divided into two decon groups, D1 and D2, with each group having 5 males and 5 females. The operating conditions for each run are summarized in Table A1.

TABLE A1. Summary of Exposure Runs for Rat VX Lethality Study Parts I and II

Date	Test Group	t (min)	C (mg/m^3)	CT (mg-min/m^3)	Study Part	Decon Groups
13-Jul-05	G18	10	3.48	34.8	I	D1 & D2
30-Jun-05	G15	10	3.65	36.5	I	D1 & D2
3-Aug-05	G22	10	4.64	46.4	I	D1 & D2
10-Aug-05	G24	10	5.73	57.3	I	D1 & D2
31-Aug-05	G27	10	6.04	60.4	I	D1 & D2
7-Sep-05	G28	10	6.80	68.0	I	D1 & D2
8-Sep-05	G29	10	7.05	70.5	I	D1 & D2
22-Jun-05	G13	60	0.523	31.4	I	D1 & D2
21-Jul-05	G20	60	0.571	34.3	I	D1 & D2
28-Jun-05	G14	60	0.667	40.0	I	D1 & D2
27-Jul-05	G21	60	0.691	41.5	I	D1 & D2
4-Aug-05	G23	60	0.775	46.5	I	D1 & D2
21-Jun-05	G12	240	0.130	31.2	I	D1 & D2
17-Aug-05	G26	240	0.133	31.9	I	D1 & D2
20-Jul-05	G19	240	0.144	34.6	I	D1 & D2
16-Aug-05	G25	240	0.156	37.4	I	D1 & D2
12-Jul-05	G17	240	0.167	40.1	I	D1 & D2
3-Nov-05	G44	10	3.28	32.8	II	ND
13-Oct-05	G35	10	4.13	41.3	II	ND
1-Nov-05	G42	10	5.18	51.8	II	ND
18-Oct-05	G38	10	5.53	55.3	II	ND
27-Oct-05	G41	10	6.35	63.5	II	ND
4-Oct-05	G31	60	0.503	30.2	II	ND
6-Oct-05	G33	60	0.594	35.6	II	ND
11-Oct-05	G34	60	0.665	39.9	II	ND
18-Oct-05	G37	60	0.673	40.4	II	ND
13-Oct-05	G36	60	0.811	48.7	II	ND
4-Oct-05	G30	240	0.138	33.1	II	ND
6-Oct-05	G32	240	0.162	38.9	II	ND
20-Oct-05	G39	240	0.172	41.2	II	ND
25-Oct-05	G40	240	0.184	44.0	II	ND
2-Nov-05	G43	240	0.195	46.8	II	ND

7 STATISTICAL ANALYSIS OF ORDINAL RESPONSE DATA FROM PART I

The following are the MINITAB® printouts for the Part I results. Examples are presented (preceded by [DRS]) on how various final parameter values (median effective dosages, dosage ratio of severe and lethal effects, etc.) are calculated from these printouts in the first section in which a particular calculation first occurs.

7.1 Female Rat (Part I, 10 Minutes)—Ordinal Response vs logCT and Dgroup

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1          9
           2          8
           3         30
           4         23
           Total     70

Logistic Regression Table

Predictor      Coef    SE Coef       Z      P
Const(1)    17.5242    3.07297    5.70  0.000
Const(2)    18.3577    3.12509    5.87  0.000
Const(3)    20.6204    3.41588    6.04  0.000
logCT      -11.4976    1.91920   -5.99  0.000
Dgroup     0.253948   0.145770    1.74  0.081

Log-Likelihood = -58.650
Test that all slopes are zero: G = 56.364, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests
Method     Chi-Square  DF      P
Pearson       34.5688  37  0.584
Deviance      36.7247  37  0.482
```

Variance-Covariance Matrix

```
Row   Const(1)   Const(2)   Const(3)    logCT      Dgroup
  1     9.4431     9.5717    10.4454  -5.87717   0.0461001
  2     9.5717     9.7662    10.6332  -5.98201   0.0486902
  3    10.4454    10.6332    11.6682  -6.54535   0.0558161
  4    -5.8772    -5.9820    -6.5453   3.68333  -0.0309426
  5     0.0461     0.0487     0.0558  -0.03094   0.0212489
```

[DRS] Examples of how LCT_{50} and ECT_{50}(severe) are calculated from results provided by MINITAB® are shown in Equations [A1] and [A2] for Dgroup = 1.

$$\log_{10}\left(LCT_{50}\right)\Big|_{\substack{Score=4\\Dgroup=1}} = \frac{\left[Z - k_{Score[4]} - (\text{Dgroup})k_{Dgroup}\right]}{k_{CT}}$$

$$= \frac{\left[0 - (20.6204) - (1)(0.25395)\right]}{(-11.4976)} = 1.8155 \qquad \text{[A1]}$$

$$\text{or } LCT_{50} = 65.4 \text{ mg-min/m}^3$$

$$\log_{10}\left(ECT_{50}\right)\Big|_{\substack{Score=3\\Dgroup=1}} = \frac{\left[Z - k_{Score[3]} - (Dgroup)k_{Dgroup}\right]}{k_{CT}}$$

$$= \frac{\left[0 - (18.3577) - (1)(0.25395)\right]}{(-11.4976)} = 1.6187 \qquad [A2]$$

or ECT_{50}(severe) = 41.6 mg-min/m^3

[DRS] Example of how the approximate 95% confidence limits for the above estimate of the LCT_{50} for Dgroup = 1 is calculated.

(1) The standard error of a ratio needs to be calculated. From Mood et al. (1974), the following is given:[1]

$$\text{var}(a/b) = \left[\frac{a^2}{b^2}\right]\left[\frac{\text{var}(a)}{a^2} + \frac{\text{var}(b)}{b^2} - \frac{(2)\text{cov}(a,b)}{ab}\right] \qquad [A3]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)}$$

For this example, a represents the numerator in either Equation [A1] or [A2], and b represents the denominator.

(2) Using values from the variance-covariance matrix, the variance of the numerator, var(num) or var(a), equals:

$$\text{var}(\text{num}) = \text{var}\left(k_{Score[4]}\right) + \text{var}\left(k_{Dgroup}\right) \pm (2)(\text{Dgroup})\text{cov}\left(k_{Score[4]},\ k_{Dgroup}\right) \qquad [A4]$$

$$\text{var}(\text{num}) = (11.6682) + (0.02125) + (2)(1)(0.05582) = 11.8011$$

(3) Using values from the variance-covariance matrix, the covariance of the numerator and the denominator, cov(num,den) or cov(a,b), equals:

$$\text{cov}(\text{num,den}) = (-1)\text{cov}\left(k_{Score[4]},\ k_{CT}\right) - (\text{Dgroup})\text{cov}\left(k_{Dgroup},\ k_{CT}\right) \qquad [A5]$$

$$\text{cov}(\text{num,den}) = (-1)(-6.5453) + (1)(-0.0309) = 6.5762$$

[1] Mood, AM, Graybill, FA, and Boes, DC, Introduction to the Theory of Statistics. Third Edition, McGraw-Hill, NY, 1974.

(4) From the variance-covariance matrix, the variance of the denominator, var(k_{CT}) or var(b), equals 3.68333.

(5) Thus, the standard error (using Equation [A3]) equals:

$$\text{var}(a/b) = \left[\frac{(-20.8743)^2}{(-11.4976)^2}\right]\left[\frac{(11.8011)}{(-20.8743)^2} + \frac{(3.6833)}{(-11.4976)^2} - \frac{(2)(6.5762)}{(-20.8743)(-11.4976)}\right]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)} = 0.0219 \qquad \text{[A6]}$$

(6) The approximate 95% confidence limits for the LCT_{50} for Dgroup = 1 now equal:

$$\hat{\mu}_j - (1.96)(\text{Stnd Err}) \le \log(LCT_{50}) \le \hat{\mu}_j + (1.96)(\text{Stnd Err})$$

or

$$\log(65.4) - (1.96)(0.0219) \le \log(LCT_{50}) \le \log(65.4) + (1.96)(0.0219) \qquad \text{[A7]}$$

$$59.3 \le LCT_{50} \le 72.2$$

7.2 Female Rat (Part I, 60 Minutes)—Ordinal Response vs logCT and Dgroup

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1          3
           2         12
           3         21
           4         14
           Total     50

Logistic Regression Table

Predictor      Coef    SE Coef       Z      P
Const(1)    17.6404    4.58435    3.85  0.000
Const(2)    18.9810    4.65345    4.08  0.000
Const(3)    20.4171    4.74997    4.30  0.000
logCT      -12.4043    2.96350   -4.19  0.000
Dgroup     0.316524   0.162567    1.95  0.052

Log-Likelihood = -51.168
Test that all slopes are zero: G = 20.873, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests
Method     Chi-Square  DF      P
Pearson       25.9527  25  0.410
Deviance      21.0824  25  0.688
```

Variance-Covariance Matrix

```
Row    Const(1)  Const(2)  Const(3)    logCT      Dgroup
  1    21.0162   21.2764   21.7035  -13.5513   0.123907
```

2	21.2764	21.6546	22.0716	-13.7768	0.129700
3	21.7035	22.0716	22.5622	-14.0627	0.136312
4	-13.5513	-13.7768	-14.0627	8.7823	-0.083329
5	0.1239	0.1297	0.1363	-0.0833	0.026428

7.3 Female Rat (Part I, 240 Minutes)—Ordinal Response vs logCT and Dgroup

Link Function: Normit

Response Information

Variable	Value	Count
Score[1d]	1	5
	2	13
	3	18
	4	14
	Total	50

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Const(1)	33.5484	7.36241	4.56	0.000
Const(2)	35.0015	7.46083	4.69	0.000
Const(3)	36.6293	7.59268	4.82	0.000
logCT	-23.0948	4.87080	-4.74	0.000
Dgroup	0.801070	0.187455	4.27	0.000

Log-Likelihood = -45.911
Test that all slopes are zero: G = 38.650, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	26.4076	25	0.386
Deviance	29.3325	25	0.250

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logCT	Dgroup
1	54.2051	54.8720	55.8189	-35.8273	0.439407
2	54.8720	55.6640	56.6029	-36.3237	0.458546
3	55.8189	56.6029	57.6487	-36.9638	0.479406
4	-35.8273	-36.3237	-36.9638	23.7247	-0.302632
5	0.4394	0.4585	0.4794	-0.3026	0.035139

7.4 Male Rat (Part I, 10 Minutes)—Ordinal Response vs logCT and Dgroup

Link Function: Normit

Response Information

Variable	Value	Count
Score[1d]	1	10
	2	17
	3	26
	4	17
	Total	70

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Const(1)	16.6729	2.66474	6.26	0.000

```
Const(2)     18.0806   2.78889   6.48  0.000
Const(3)     19.8289   2.94020   6.74  0.000
logCT       -10.8354   1.64584  -6.58  0.000
Dgroup      0.355979  0.141887   2.51  0.012
Log-Likelihood = -64.557
Test that all slopes are zero: G = 57.545, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       42.5047  37  0.246
Deviance      45.2611  37  0.165
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)   Const(3)    logCT       Dgroup
  1    7.10084    7.39412    7.78660   -4.36785    0.0775539
  2    7.39412    7.77793    8.16671   -4.57842    0.0822403
  3    7.78660    8.16671    8.64476   -4.82794    0.0900565
  4   -4.36785   -4.57842   -4.82794    2.70880   -0.0495224
  5    0.07755    0.08224    0.09006   -0.04952    0.0201320
```

7.5 Male Rat (Part I, 60 Minutes)—Ordinal Response vs logCT and Dgroup

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1          3
           2         16
           3         17
           4         14
           Total     50

Logistic Regression Table

Predictor      Coef   SE Coef      Z      P
Const(1)    11.6363   4.22832   2.75  0.006
Const(2)    13.0801   4.28708   3.05  0.002
Const(3)    14.1294   4.33121   3.26  0.001
logCT      -8.49924   2.71334  -3.13  0.002
Dgroup     0.300784  0.158919   1.89  0.058

Log-Likelihood = -55.965
Test that all slopes are zero: G = 13.735, DF = 2, P-Value = 0.001

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       16.1612  25  0.910
Deviance      19.2189  25  0.787
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)   Const(3)    logCT       Dgroup
  1    17.8787    18.0757    18.2504   -11.4419    0.0122944
  2    18.0757    18.3791    18.5449   -11.6206    0.0183832
  3    18.2504    18.5449    18.7594   -11.7394    0.0225129
  4   -11.4419   -11.6206   -11.7394    7.3622    -0.0131889
```

5	0.0123	0.0184	0.0225	-0.0132	0.0252552

7.6 Male Rat (Part I, 240 Minutes)—Ordinal Response vs logCT and Dgroup

Link Function: Normit

Response Information

Variable	Value	Count
Score[1d]	1	6
	2	8
	3	21
	4	15
	Total	50

Logistic Regression Table

Predictor	Coef	SE Coef	Z	P
Const(1)	28.8517	6.90071	4.18	0.000
Const(2)	29.6677	6.93912	4.28	0.000
Const(3)	31.1580	7.04891	4.42	0.000
logCT	-19.7388	4.53706	-4.35	0.000
Dgroup	0.480168	0.168598	2.85	0.004

Log-Likelihood = -50.317
Test that all slopes are zero: G = 26.685, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	33.8949	25	0.110
Deviance	36.6949	25	0.062

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logCT	Dgroup
1	47.6198	47.8538	48.5891	-31.2857	0.217923
2	47.8538	48.1514	48.8775	-31.4680	0.223085
3	48.5891	48.8775	49.6871	-31.9663	0.234030
4	-31.2857	-31.4680	-31.9663	20.5849	-0.148609
5	0.2179	0.2231	0.2340	-0.1486	0.028425

7.7 Combined Male and Female Rat (Part I, 10 Minutes)—Ordinal Response vs logCT, Dgroup and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 10 minute exposures.

Link Function: Normit

Response Information

Variable	Value	Count
Score[1d]	1	19
	2	25
	3	56
	4	40
	Total	140

```
Logistic Regression Table
Predictor       Coef    SE Coef       Z      P
Const(1)     16.7205    1.94222    8.61  0.000
Const(2)     17.8619    2.00507    8.91  0.000
Const(3)     19.8076    2.14436    9.24  0.000
logCT       -10.9306    1.20355   -9.08  0.000
Dgroup       0.310798   0.101192   3.07  0.002 [DRS] Dgroup is statistically significant.
Gender       0.232368   0.100364   2.32  0.021 [DRS] Gender is statistically significant.

Log-Likelihood = -124.877
Test that all slopes are zero: G = 115.124, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests
Method     Chi-Square  DF      P
Pearson       83.0783  78  0.326
Deviance      85.3264  78  0.267
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logCT	Dgroup	Gender
1	3.77222	3.87645	4.14007	-2.32803	0.0317942	0.0241108
2	3.87645	4.02030	4.28148	-2.40665	0.0336248	0.0254679
3	4.14007	4.28148	4.59829	-2.57550	0.0374539	0.0284276
4	-2.32803	-2.40665	-2.57550	1.44854	-0.0206900	-0.0156980
5	0.03179	0.03362	0.03745	-0.02069	0.0102398	0.0004606
6	0.02411	0.02547	0.02843	-0.01570	0.0004606	0.0100729

[DRS] Example on how the ratio of median effective dosages (female to male) are calculated from values shown in the MINITAB® printouts is shown below.

$$\log_{10}\left(\frac{\text{E or LCT}_{50}(\text{male})}{\text{E or LCT}_{50}(\text{female})}\right) = \frac{\left((-1)-1\right)k_{Gender}}{k_{CT}}$$

$$= \frac{(-2)(0.2324)}{(-10.9306)} = 0.0425 \qquad \text{[A8]}$$

$$\text{or Ratio} = 1.103$$

[DRS] Example of how the approximate 95% confidence limits for the above estimate of the ratio of the median effective dosages (male to female) is below.

(1) The standard error of a ratio needs to be calculated. From Mood et al. (1974), the following is given:[2]

[2] Mood, AM, Graybill, FA, and Boes, DC, Introduction to the Theory of Statistics. Third Edition, McGraw-Hill, NY, 1974.

$$\text{var}(a/b) = \left[\frac{a^2}{b^2}\right]\left[\frac{\text{var}(a)}{a^2} + \frac{\text{var}(b)}{b^2} - \frac{(2)\text{cov}(a,b)}{ab}\right]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)} \qquad \text{[A3]}$$

For this example, a represents the numerator in Equation [A8], and b represents the denominator.

(2) Using values from the variance-covariance matrix, the variance of the numerator, var(num) or var(a), will equal var(2 k_{Gender}). This is equivalent to 4 x var(k_{Gender}), which equals (4)(0.010073).

(3) Using values from the variance-covariance matrix, the covariance of the numerator and the denominator, cov(num,den) or cov(a,b),will equal cov(2 k_{Gender}, k_{CT}). This is equivalent to 2 x cov(k_{Gender}, k_{CT}), which equals (2)(-0.015698).

(4) From the variance-covariance matrix, the variance of the denominator, var(k_{CT}) or var(b), equals 1.448540.

(5) Thus, the standard error (using Equation [A3]) equals:

$$\text{var}(a/b) = \left[\frac{((2)(0.2324))^2}{(-10.9306)^2}\right]\left[\frac{(4)(0.010073)}{((2)(0.2324))^2} + \frac{(1.44854)}{(-10.9306)^2} + \frac{(2)(2)(-0.015698)}{((2)(0.2324))(-10.9306)}\right]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)} = 0.0184 \qquad \text{[A9]}$$

(6) The approximate 95% confidence limits for the ratio of median effective dosages for male to female now equal:

$$\hat{\mu}_j - (1.96)(\text{Stnd Err}) \le \log(\text{Ratio}) \le \hat{\mu}_j + (1.96)(\text{Stnd Err})$$

or

$$\log(1.103) - (1.96)(0.0184) \le \log(1.103) \le \log(1.103) + (1.96)(0.0184) \qquad \text{[A7]}$$

$$1.02 \le \text{Ratio} \le 1.20$$

The above procedures were also used to calculate the ratio of median effective dosages between decon groups D1 and D2 and the associated approximate 95% confidence limits.

7.8 Combined Male and Female Rat (Part I, 60 Minutes)—Ordinal Response vs logCT, Dgroup and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 60 minute exposures.

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1          6
           2         28
           3         38
           4         28
           Total    100

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     14.4344    3.07888    4.69  0.000
Const(2)     15.8159    3.12460    5.06  0.000
Const(3)     17.0423    3.17073    5.37  0.000
logCT       -10.3089    1.98280   -5.20  0.000
Dgroup       0.297608   0.112958   2.63  0.008 [DRS] Dgroup is significant.
Gender     0.0426569   0.111055   0.38  0.701 [DRS] Gender is not significant.

Log-Likelihood = -108.043
Test that all slopes are zero: G = 33.783, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       40.2581  54  0.918
Deviance      42.1206  54  0.880
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logCT	Dgroup	Gender
1	9.47950	9.59365	9.7289	-6.08871	0.0312170	0.0043848
2	9.59365	9.76311	9.8936	-6.18920	0.0341029	0.0049282
3	9.72894	9.89364	10.0535	-6.28037	0.0365866	0.0052378
4	-6.08871	-6.18920	-6.2804	3.93151	-0.0222152	-0.0031713
5	0.03122	0.03410	0.0366	-0.02222	0.0127595	-0.0004195
6	0.00438	0.00493	0.0052	-0.00317	-0.0004195	0.0123333

7.9 Combined Male and Female Rat (Part I, 240 Minutes)—Ordinal Response vs logCT, Dgroup and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 240 minute exposures.

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1         11
           2         21
           3         39
```

```
           4         29
           Total    100

Logistic Regression Table
Predictor        Coef    SE Coef       Z      P
Const(1)      30.4925    4.97731    6.13  0.000
Const(2)      31.6188    5.02115    6.30  0.000
Const(3)      33.1402    5.10322    6.49  0.000
logCT        -20.9457    3.28044   -6.39  0.000
Dgroup        0.628903   0.123878   5.08  0.000 [DRS] Dgroup is significant.
Gender      -0.0598113   0.114682  -0.52  0.602 [DRS] Gender is not significant.
Log-Likelihood = -97.782
Test that all slopes are zero: G = 63.785, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests
Method     Chi-Square  DF      P
Pearson       64.8508  54  0.148
Deviance      69.1355  54  0.080
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logCT	Dgroup	Gender
1	24.7736	24.9703	25.3678	-16.3139	0.151064	-0.0148108
2	24.9703	25.2120	25.6046	-16.4638	0.156196	-0.0152887
3	25.3678	25.6046	26.0428	-16.7326	0.163603	-0.0159870
4	-16.3139	-16.4638	-16.7326	10.7613	-0.103589	0.0101336
5	0.1511	0.1562	0.1636	-0.1036	0.015346	-0.0003039
6	-0.0148	-0.0153	-0.0160	0.0101	-0.000304	0.0131519

7.10 Combined Male and Female Rat (Part I, 10, 60 and 240 Minutes)—Ordinal Response vs logC, logT, and Dgroup

[DRS] The purpose of this analysis is to calculate the toxic load exponent for the Part I rats.

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1         36
           2         74
           3        133
           4         97
           Total    340

Logistic Regression Table
Predictor      Coef     SE Coef       Z      P
Const(1)    16.4854     1.49702   11.01  0.000
Const(2)    17.6141     1.52571   11.54  0.000
Const(3)    19.0545     1.56269   12.19  0.000
logC       -9.94909    0.837242  -11.88  0.000
logT       -11.2176    0.946228  -11.86  0.000
Dgroup     0.373989   0.0629254    5.94  0.000

Log-Likelihood = -350.071
Test that all slopes are zero: G = 180.200, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       186.770  96  0.000
```

Deviance	175.425	96	0.000

Variance-Covariance Matrix

Row	Const(1)	Const(2)	Const(3)	logC	logT	Dgroup
1	2.24107	2.27764	2.33046	-1.24172	-1.41267	0.0138360
2	2.27764	2.32778	2.37921	-1.26668	-1.44161	0.0146742
3	2.33046	2.37921	2.44199	-1.29800	-1.47660	0.0158520
4	-1.24172	-1.26668	-1.29800	0.70097	0.78717	-0.0083250
5	-1.41267	-1.44161	-1.47660	0.78717	0.89535	-0.0093602
6	0.01384	0.01467	0.01585	-0.00833	-0.00936	0.0039596

[DRS] Sample calculation of toxic load exponent and appropriate 95% confidence intervals.

(1) Calculation of toxic load exponent:

$$n = \frac{k_C}{k_T} = \frac{(-9.94909)}{(-11.2176)} = 0.0425 \qquad \text{[A10]}$$

(2) The standard error of a ratio needs to be calculated. From Mood et al. (1974), the following is given:[3]

$$\text{var}(a/b) = \left[\frac{a^2}{b^2}\right]\left[\frac{\text{var}(a)}{a^2} + \frac{\text{var}(b)}{b^2} - \frac{(2)\text{cov}(a,b)}{ab}\right]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)} \qquad \text{[A3]}$$

(3) Using values from the variance-covariance matrix, the variance of the numerator, var(num) or var(k_C), will equal 0.70097. For k_T, the variance equals 0.89535.

(4) Using values from the variance-covariance matrix, the covariance of the numerator and the denominator, cov(k_C, k_T), equals 0.78717.

(5) Thus, the standard error (using Equation [A3]) equals:

$$\text{var}(a/b) = \left[\frac{(-9.94909)^2}{(-11.2176)^2}\right]\left[\frac{(0.70097)}{(-9.94909)^2} + \frac{(0.89535)}{(-11.2176)^2} - \frac{(2)(0.78717)}{(-9.94909)(-11.2176)}\right]$$

$$\text{Stnd Error} = \sqrt{\text{var}(a/b)} = 0.0084 \qquad \text{[A11]}$$

(6) The approximate 95% confidence limits for the toxic load exponent now equal:

[3] Mood, AM, Graybill, FA, and Boes, DC, Introduction to the Theory of Statistics. Third Edition, McGraw-Hill, NY, 1974.

$$\hat{\mu}_j - (1.96)(\text{Stnd Err}) \leq n \leq \hat{\mu}_j + (1.96)(\text{Stnd Err})$$

or

$$0.887 - (1.96)(0.0084) \leq 0.887 \leq 0.887 + (1.96)(0.0084) \qquad [A12]$$

$$0.870 \leq \text{Ratio} \leq 0.903$$

7.11 Combined Male and Female Rat (Part I, 10, 60 and 240 Minutes)—Ordinal Response vs logCT and Igroup

[DRS] Calculation of an overall estimate of the probit slope (k_{CT}) for Part I rats.

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  1         36
           2         74
           3        133
           4         97
           Total    340

Factor Information
Factor  Levels  Values
Igroup      12  F240-D1, F60-D1, F10-D1, F240-D2, F60-D2, F10-D2, M10-D2, M60-D2,
                M240-D2, M10-D1, M60-D1, M240-D1

Logistic Regression Table

Predictor         Coef   SE Coef       Z      P
Const(1)       15.3129   1.32955   11.52  0.000
Const(2)       16.4967   1.36155   12.12  0.000
Const(3)       17.9905   1.40391   12.81  0.000
logCT         -10.6217  0.868934  -12.22  0.000
Igroup
  F60-D1   -0.0727111  0.314067   -0.23  0.817
  F10-D1      1.09004  0.319431    3.41  0.001
 F240-D2     -1.39305  0.321970   -4.33  0.000
  F60-D2    -0.690436  0.313060   -2.21  0.027
  F10-D2     0.630457  0.320131    1.97  0.049
  M10-D2     0.941393  0.318520    2.96  0.003
  M60-D2    -0.601425  0.319248   -1.88  0.060
 M240-D2     -1.30638  0.320003   -4.08  0.000
  M10-D1      1.60027  0.326505    4.90  0.000
  M60-D1    0.0321297  0.306925    0.10  0.917
 M240-D1    -0.297058  0.307737   -0.97  0.334

Tests for terms with more than 1 degree of freedom

Term     Chi-Square  DF      P
Igroup      113.437  11  0.000

Log-Likelihood = -340.779
Test that all slopes are zero: G = 198.783, DF = 12, P-Value = 0.000

Goodness-of-Fit Tests
```

```
Method      Chi-Square   DF      P
Pearson        210.817  189  0.132
Deviance       216.737  189  0.081
```

8 STATISTICAL ANALYSIS OF ORDINAL RESPONSE DATA FROM PART II

The following are the MINITAB® printouts for the Part II results.

8.1 Female Rat (Part II, 10 Minutes)—Ordinal Response vs logCT

[DRS] An outlier was identified from the run with vapor concentration of 5.5 mg/m^3 from 18 October 2005. So, the following analyzes in this section are done both with and without response data from this run.

8.1.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2         15
           3         17
           4         18
           Total     50

Logistic Regression Table

Predictor       Coef  SE Coef       Z      P
Const(1)     17.7534  3.57311    4.97  0.000
Const(2)     19.1255  3.70472    5.16  0.000
logCT       -11.0187  2.15837   -5.11  0.000

Log-Likelihood = -38.887
Test that all slopes are zero: G = 31.804, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

Method      Chi-Square  DF      P
Pearson        18.5557   7  0.010
Deviance       18.9194   7  0.008
```

Variance-Covariance Matrix

```
Row  Const(1)  Const(2)    logCT
  1   12.7671  13.2006  -7.69596
  2   13.2006  13.7249  -7.98297
  3   -7.6960  -7.9830   4.65857
```

8.1.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
```

```
Score[1d]wo  2          12
             3          12
             4          16
             Total      40

Logistic Regression Table

Predictor       Coef  SE Coef       Z       P
Const(1)     25.2442  5.05435    4.99   0.000
Const(2)     27.1616  5.37787    5.05   0.000
logCT       -15.8996  3.15328   -5.04   0.000
Log-Likelihood = -22.568
Test that all slopes are zero: G = 41.976, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests
Method     Chi-Square  DF       P
Pearson      6.26584    5   0.281
Deviance     6.87408    5   0.230
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)    logCT
  1     25.5464    27.1055  -15.9101
  2     27.1055    28.9215  -16.9336
  3    -15.9101   -16.9336    9.9431
```

8.2 Female Rat (Part II, 60 Minutes)—Ordinal Response vs logCT

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2          9
           3         26
           4         15
           Total     50

Logistic Regression Table

Predictor       Coef  SE Coef       Z       P
Const(1)     15.5621  4.27798    3.64   0.000
Const(2)     17.3479  4.39754    3.94   0.000
logCT       -10.5335  2.74315   -3.84   0.000

Log-Likelihood = -42.269
Test that all slopes are zero: G = 16.452, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF       P
Pearson      5.80765    7   0.562
Deviance     6.31073    7   0.504
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)    logCT
  1     18.3011    18.7744  -11.7183
  2     18.7744    19.3384  -12.0502
  3    -11.7183   -12.0502    7.5249
```

8.3 Female Rat (Part II, 240 Minutes)—Ordinal Response vs logCT

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  2          6
           3         16
           4         27
           Total     49
Logistic Regression Table
Predictor       Coef  SE Coef       Z      P
Const(1)     19.9848  5.71942    3.49  0.000
Const(2)     21.2651  5.80554    3.66  0.000
logCT       -13.3325  3.61381   -3.69  0.000

Log-Likelihood = -39.098
Test that all slopes are zero: G = 15.003, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests
Method    Chi-Square  DF      P
Pearson      9.54062   7  0.216
Deviance     9.24363   7  0.236
```

Variance-Covariance Matrix

```
Const(1)  Const(2)   logCT
 32.7117   33.1666  -20.6475
 33.1666   33.7043  -20.9683
-20.6475  -20.9683   13.0596
```

8.4 Male Rat (Part II, 10 Minutes)—Ordinal Response vs logCT

[DRS] An outlier was identified from the run with vapor concentration of 5.5 mg/m^3 from 18 October 2005. So, the following analyzes in this section are done both with and without response data from this run.

8.4.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  2         10
           3         15
           4         25
           Total     50

Logistic Regression Table
Predictor       Coef  SE Coef       Z      P
Const(1)     12.9038  3.09520    4.17  0.000
Const(2)     14.0701  3.18495    4.42  0.000
logCT       -8.34629  1.88659   -4.42  0.000

Log-Likelihood = -40.822
Test that all slopes are zero: G = 21.321, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests
```

```
Method      Chi-Square  DF      P
Pearson        22.8487   7  0.002
Deviance       25.7647   7  0.001
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)    logCT
  1     9.58025    9.8265  -5.82288
  2     9.82653   10.1439  -5.99703
  3    -5.82288   -5.9970   3.55921
```

8.4.2 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
Score[1d]wo   2          8
              3          9
              4         23
              Total     40

Logistic Regression Table

Predictor       Coef  SE Coef       Z      P
Const(1)     21.4296  4.77717    4.49  0.000
Const(2)     22.8710  4.97126    4.60  0.000
logCT       -13.9101  3.02223   -4.60  0.000

Log-Likelihood = -22.463
Test that all slopes are zero: G = 33.131, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

Method      Chi-Square  DF      P
Pearson        9.36950   5  0.095
Deviance       8.05091   5  0.153
```

Variance-Covariance Matrix

```
Row    Const(1)   Const(2)    logCT
  1     22.8213    23.6850  -14.4094
  2     23.6850    24.7134  -15.0016
  3    -14.4094   -15.0016    9.1339
```

8.5 Male Rat (Part II, 60 Minutes)—Ordinal Response vs logCT

```
Link Function: Normit

Response Information

Variable    Value  Count
Score[1d]   2          9
            3         18
            4         23
            Total     50

Logistic Regression Table
Predictor       Coef  SE Coef       Z      P
```

```
Const(1)     21.2799  4.84873   4.39  0.000
Const(2)     22.6979  4.96352   4.57  0.000
logCT       -14.2442  3.12524  -4.56  0.000

Log-Likelihood = -39.157
Test that all slopes are zero: G = 25.053, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       7.10279   7  0.418
Deviance      7.03148   7  0.426
```

Variance-Covariance Matrix

```
Row   Const(1)  Const(2)   logCT
  1   23.5102   24.0288  -15.1342
  2   24.0288   24.6365  -15.4991
  3  -15.1342  -15.4991    9.7671
```

8.6 Male Rat (Part II, 240 Minutes)—Ordinal Response vs logCT

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2          6
           3         17
           4         27
           Total     50

Logistic Regression Table

Predictor      Coef  SE Coef      Z      P
Const(1)    14.9320  5.33914   2.80  0.005
Const(2)    16.1621  5.39864   2.99  0.003
logCT      -10.1161  3.35675  -3.01  0.003

Log-Likelihood = -42.999
Test that all slopes are zero: G = 9.398, DF = 1, P-Value = 0.002

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson      13.4172   7  0.063
Deviance     13.2188   7  0.067
```

Variance-Covariance Matrix

```
Row   Const(1)  Const(2)   logCT
  1   28.5064   28.7913  -17.9030
  2   28.7913   29.1453  -18.1112
  3  -17.9030  -18.1112   11.2678
```

8.7 Combined Male and Female Rat (Part II, 10 Minutes)—Ordinal Response vs logCT and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 10 minute exposures.

8.7.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information
Variable   Value  Count
Score[1d]  2         25
           3         32
           4         43
           Total    100
Logistic Regression Table

Predictor       Coef   SE Coef      Z      P
Const(1)     15.1110   2.32405   6.50  0.000
Const(2)     16.3741   2.40159   6.82  0.000
logCT       -9.56056   1.41167  -6.77  0.000
Gender     -0.241790  0.124393  -1.94  0.052

Log-Likelihood = -80.158
Test that all slopes are zero: G = 54.505, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      42.7564  16  0.000
Deviance     45.5806  16  0.000
```

Variance-Covariance Matrix

```
Row   Const(1)  Const(2)   logCT     Gender
  1   5.40123   5.56452  -3.27275  -0.0360988
  2   5.56452   5.76762  -3.38405  -0.0385474
  3  -3.27275  -3.38405   1.99282   0.0226636
  4  -0.03610  -0.03855   0.02266   0.0154737
```

8.7.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
Score[1d]wo   2         20
              3         21
              4         39
              Total     80

Logistic Regression Table

Predictor       Coef   SE Coef      Z      P
Const(1)     23.2834   3.45414   6.74  0.000
Const(2)     24.9583   3.63454   6.87  0.000
logCT       -14.8857   2.17073  -6.86  0.000
Gender     -0.416182  0.163521  -2.55  0.011

Log-Likelihood = -45.276
Test that all slopes are zero: G = 77.115, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
```

```
Pearson        17.6891  12  0.125
Deviance       15.4161  12  0.219
```

Variance-Covariance Matrix

```
Row   Const(1)   Const(2)    logCT      Gender
  1    11.9311   12.5191   -7.48398   -0.165980
  2    12.5191   13.2099   -7.87785   -0.176080
  3    -7.4840   -7.8778    4.71205    0.105589
  4    -0.1660   -0.1761    0.10559    0.026739
```

8.8 Combined Male and Female Rat (Part II, 60 Minutes)—Ordinal Response vs logCT and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 60 minute exposures.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2         18
           3         44
           4         38
           Total    100

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     18.0261    3.16172    5.70  0.000
Const(2)     19.6296    3.24406    6.05  0.000
logCT       -12.1344    2.03191   -5.97  0.000
Gender     -0.172711   0.120108   -1.44  0.150

Log-Likelihood = -82.691
Test that all slopes are zero: G = 42.133, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       15.0428  16  0.522
Deviance      15.8739  16  0.462
```

Variance-Covariance Matrix

```
Row   Const(1)   Const(2)    logCT      Gender
  1     9.9965   10.2378   -6.41533   -0.0290727
  2    10.2378   10.5240   -6.58530   -0.0313907
  3    -6.4153   -6.5853    4.12865    0.0194856
  4    -0.0291   -0.0314    0.01949    0.0144260
```

8.9 Combined Male and Female Rat (Part II, 240 Minutes)—Ordinal Response vs logCT and Gender

[DRS] The purpose of this analysis is to determine whether gender effects are statistically significant for the 240 minute exposures.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2         12
           3         33
           4         54
           Total     99

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     17.3456    3.89433    4.45  0.000
Const(2)     18.5961    3.94489    4.71  0.000
logCT       -11.6497    2.45418   -4.75  0.000
Gender     0.0276059   0.122104    0.23  0.821

Log-Likelihood = -82.312
Test that all slopes are zero: G = 23.992, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      23.6490  16  0.097
Deviance     22.8921  16  0.117
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	logCT	Gender
1	15.1658	15.3452	-9.54731	0.0093031
2	15.3452	15.5621	-9.67584	0.0095878
3	-9.5473	-9.6758	6.02298	-0.0060852
4	0.0093	0.0096	-0.00609	0.0149093

8.10 Combined Male and Female Rat (Part II, 10, 60 and 240 Minutes)—Ordinal Response vs logC, logT, and Gender

[DRS] The purpose of this analysis is to calculate the toxic load exponent for the Part II rats.

8.10.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2         55
           3        109
           4        135
           Total    299

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     16.0581    1.65335    9.71  0.000
Const(2)     17.4070    1.69257   10.28  0.000
logC        -9.71259   0.967377  -10.04  0.000
logT        -10.5544    1.03185  -10.23  0.000
Gender     -0.124719  0.0697627   -1.79  0.074
```

```
Log-Likelihood = -250.707
Test that all slopes are zero: G = 119.507, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       100.547  55  0.000
Deviance       95.438  55  0.001
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	logC	logT	Gender
1	2.73356	2.79269	-1.58348	-1.70307	-0.0059358
2	2.79269	2.86480	-1.62151	-1.74446	-0.0063802
3	-1.58348	-1.62151	0.93582	0.99070	0.0035945
4	-1.70307	-1.74446	0.99070	1.06472	0.0038834
5	-0.00594	-0.00638	0.00359	0.00388	0.0048668

8.10.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
Score[1d]wo   2         50
              3         98
              4        131
              Total    279

Logistic Regression Table

Predictor        Coef    SE Coef       Z      P
Const(1)      19.3389    1.87351   10.32  0.000
Const(2)      20.7827    1.92344   10.80  0.000
logC         -12.0642    1.14067  -10.58  0.000
logT         -12.7200    1.18230  -10.76  0.000
Gender     -0.142336  0.0743892   -1.91  0.056

Log-Likelihood = -215.590
Test that all slopes are zero: G = 143.878, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       71.5598  51  0.030
Deviance      64.8036  51  0.093
```

Variance-Covariance Matrix

Row	Const(1)	Const(2)	logC	logT	Gender
1	3.51003	3.59660	-2.11541	-2.21146	-0.0096149
2	3.59660	3.69962	-2.17200	-2.27153	-0.0102473
3	-2.11541	-2.17200	1.30113	1.33905	0.0060096
4	-2.21146	-2.27153	1.33905	1.39784	0.0062983
5	-0.00961	-0.01025	0.00601	0.00630	0.0055337

8.11 Combined Male and Female Rat (Part II, 10, 60 and 240 Minutes)—Ordinal Response vs logCT and Igroup

[DRS] The following is a calculation of an overall estimate of the probit slope (k_{CT}) for Part II rats.

8.11.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  2         55
           3        109
           4        135
           Total    299

Factor Information

Factor   Levels  Values
GTgroup       6  F10, M10, F60, M60, F240, M240

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     17.1436    1.75171    9.79  0.000
Const(2)     18.5164    1.79209   10.33  0.000
logCT       -10.6579    1.04694  -10.18  0.000
GTgroup
 M10       -0.513382   0.251534   -2.04  0.041
 F60        -1.14973   0.263569   -4.36  0.000
 M60        -1.47441   0.269833   -5.46  0.000
 F240       -1.49296   0.263960   -5.66  0.000
 M240       -1.44394   0.261481   -5.52  0.000

Tests for terms with more than 1 degree of freedom

Term     Chi-Square  DF      P
GTgroup     47.2864   5  0.000

Log-Likelihood = -246.599
Test that all slopes are zero: G = 127.723, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      88.4397  52  0.001
Deviance     87.2216  52  0.002
```

8.11.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable     Value  Count
Score[1d]wo  2         50
             3         98
             4        131
```

```
            Total    279

Factor Information

Factor   Levels  Values
GTgroup       6  F10, M10, F60, M60, F240, M240

Logistic Regression Table

Predictor       Coef   SE Coef       Z      P
Const(1)     20.5058   1.98341   10.34  0.000
Const(2)     21.9752   2.03529   10.80  0.000
logCT       -12.8931   1.20796  -10.67  0.000
GTgroup
 M10       -0.743884  0.304835   -2.44  0.015
 F60        -1.03162  0.282507   -3.65  0.000
 M60        -1.37909  0.289156   -4.77  0.000
 F240       -1.34982  0.283375   -4.76  0.000
 M240       -1.29549  0.280993   -4.61  0.000

Tests for terms with more than 1 degree of freedom

Term     Chi-Square  DF      P
GTgroup     31.3091   5  0.000

Log-Likelihood = -211.799
Test that all slopes are zero: G = 151.460, DF = 6, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      58.9876  48  0.133
Deviance     57.2212  48  0.170
```

9 STATISTICAL ANALYSIS OF ORDINAL RESPONSE DATA FROM PARTS I AND II

The following are the MINITAB® printouts for the analysis of the combined data from Parts I and Part II. Examples are presented (preceded by [DRS]) on how various final parameter values (median effective dosages, dosage ratio of severe and lethal effects, etc.) are calculated from these printouts in the first section in which a particular calculation first occurs.

9.1 Combined Male and Female Rat (Parts I and II, 10 Minutes)—Ordinal Response vs logCT and Dgrp

[DRS] The purpose of this analysis is to determine whether decon group effects are statistically significant for the 10 minute exposures. Gender effects were previously determined to be negligible when all three decon groups are examined together.

9.1.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1         19
```

```
2          50
3          88
4          83
Total     240

Factor Information

Factor  Levels  Values
Dgrp         3  D1, D2, ND

Logistic Regression Table

Predictor       Coef   SE Coef       Z      P
Const(1)     15.2210   1.42718   10.67  0.000
Const(2)     16.6884   1.48503   11.24  0.000
Const(3)     18.2421   1.55570   11.73  0.000
logCT       -9.91934  0.870125  -11.40  0.000
Dgrp
 D2         -0.574285  0.196080   -2.93  0.003
 ND          -1.23233  0.192932   -6.39  0.000

Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Dgrp     41.1206   2  0.000

Log-Likelihood = -218.407
Test that all slopes are zero: G = 169.263, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       129.967  51  0.000
Deviance      124.167  51  0.000
```

9.1.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
Score[1d]wo   1         19
              2         45
              3         77
              4         79
              Total    220

Factor Information

Factor  Levels  Values
Dgrp         3  D1, D2, ND

Logistic Regression Table
Predictor       Coef   SE Coef       Z      P
Const(1)     18.0386   1.63633   11.02  0.000
Const(2)     19.5484   1.70385   11.47  0.000
Const(3)     21.3014   1.80101   11.83  0.000
logCT       -11.6321   1.00369  -11.59  0.000
Dgrp
 D2         -0.620525  0.200439   -3.10  0.002
 ND          -1.73072  0.225182   -7.69  0.000
```

```
Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Dgrp     59.7434   2  0.000
Log-Likelihood = -183.523
Test that all slopes are zero: G = 192.343, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       90.7144  48  0.000
Deviance      94.2897  48  0.000
```

9.2 Combined Male and Female Rat (Parts I and II, 60 Minutes)—Ordinal Response vs logCT and Dgrp

[DRS] The purpose of this analysis is to determine whether decon group effects are statistically significant for the 60 minute exposures. Gender effects were previously determined to be negligible when all three decon groups are examined together.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1          6
           2         46
           3         82
           4         66
           Total    200

Factor Information

Factor  Levels  Values
Dgrp         3  D1, D2, ND

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     15.5561    2.17792    7.14  0.000
Const(2)     17.1151    2.21461    7.73  0.000
Const(3)     18.5081    2.25796    8.20  0.000
logCT       -10.9558    1.39907   -7.83  0.000
Dgrp
 D2         -0.646812   0.226341  -2.86  0.004
 ND         -0.848052   0.199449  -4.25  0.000

Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Dgrp     18.3418   2  0.000

Log-Likelihood = -194.829
Test that all slopes are zero: G = 80.197, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       41.2157  39  0.374
Deviance      33.5734  39  0.715
```

9.3 Combined Male and Female Rat (Parts I and II, 240 Minutes)—Ordinal Response vs logCT and Dgrp

[DRS] The purpose of this analysis is to determine whether decon group effects are statistically significant for the 240 minute exposures. Gender effects were previously determined to be negligible when all three decon groups are examined together.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1         13
           2         31
           3         72
           4         83
           Total    199

Factor Information

Factor  Levels  Values
Dgrp         3  D1, D2, ND

Logistic Regression Table

Predictor       Coef    SE Coef       Z      P
Const(1)     21.7266    2.96731    7.32  0.000
Const(2)     22.7426    2.98995    7.61  0.000
Const(3)     24.0989    3.03476    7.94  0.000
logCT       -14.7761    1.93848   -7.62  0.000
Dgrp
 D2          -1.13239   0.232589  -4.87  0.000
 ND         -0.472045   0.220939  -2.14  0.033

Tests for terms with more than 1 degree of freedom

Term  Chi-Square  DF      P
Dgrp     23.7325   2  0.000

Log-Likelihood = -189.240
Test that all slopes are zero: G = 99.292, DF = 3, P-Value = 0.000

Goodness-of-Fit Tests

Method    Chi-Square  DF      P
Pearson      87.1314  39  0.000
Deviance     87.0721  39  0.000
```

9.4 Combined Male and Female Rat (Part II, 10, 60 and 240 Minutes)—Ordinal Response vs logCT and Igroup

[DRS] The following is a calculation of an overall estimate of the probit slope (k_{CT}) for Parts I and II rats combined.

9.4.1 Analysis with Outlier Run

```
Link Function: Normit
```

```
Response Information

Variable   Value  Count
Score[1d]  1         38
           2        127
           3        242
           4        232
           Total    639

Factor Information

Factor  Levels  Values
Igroup      18  F10-D1, F10-D2, F10-ND, M10-D1, M10-D2, M10-ND, F60-D1, F60-D2,
                F60-ND, M60-D1, M60-D2, M60-ND, F240-D1, F240-D2, F240-ND,
                M240-D1, M240-D2, M240-ND

Logistic Regression Table

Predictor        Coef    SE Coef       Z      P
Const(1)      15.8161    1.10153   14.36  0.000
Const(2)      17.1747    1.12867   15.22  0.000
Const(3)      18.6055    1.15631   16.09  0.000
logCT        -10.3375   0.655542  -15.77  0.000
Igroup
 F10-D2      -0.466676   0.280392   -1.66  0.096
 F10-ND      -0.753992   0.256720   -2.94  0.003
 M10-D1       0.501387   0.273485    1.83  0.067
 M10-D2      -0.149147   0.276237   -0.54  0.589
 M10-ND       -1.19278   0.264385   -4.51  0.000
 F60-D1       -1.15046   0.306652   -3.75  0.000
 F60-D2       -1.75674   0.308791   -5.69  0.000
 F60-ND       -1.76164   0.268327   -6.57  0.000
 M60-D1       -1.03255   0.300201   -3.44  0.001
 M60-D2       -1.68404   0.313651   -5.37  0.000
 M60-ND       -2.07591   0.273847   -7.58  0.000
 F240-D1      -1.04506   0.307734   -3.40  0.001
 F240-D2      -2.45436   0.324330   -7.57  0.000
 F240-ND      -2.01356   0.271479   -7.42  0.000
 M240-D1      -1.33881   0.309101   -4.33  0.000
 M240-D2      -2.37876   0.322643   -7.37  0.000
 M240-ND      -2.04231   0.271081   -7.53  0.000

Tests for terms with more than 1 degree of freedom

Term     Chi-Square  DF      P
Igroup      196.375  17  0.000

Log-Likelihood = -605.640
Test that all slopes are zero: G = 353.665, DF = 18, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square   DF      P
Pearson       423.403  273  0.000
Deviance      340.484  273  0.003
```

9.4.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information
```

```
Variable      Value  Count
Score[1d]wo   1         38
              2        122
              3        231
              4        228
              Total    619

Factor Information

Factor  Levels  Values
Igroup      18  F10-D1, F10-D2, F10-ND, M10-D1, M10-D2, M10-ND, F60-D1, F60-D2,
                F60-ND, M60-D1, M60-D2, M60-ND, F240-D1, F240-D2, F240-ND,
                M240-D1, M240-D2, M240-ND

Logistic Regression Table

Predictor        Coef    SE Coef       Z      P
Const(1)      17.3341    1.16731   14.85  0.000
Const(2)      18.7004    1.19603   15.64  0.000
Const(3)      20.1756    1.22648   16.45  0.000
logCT        -11.2446   0.696139  -16.15  0.000
Igroup
 F10-D2     -0.474810   0.283947   -1.67  0.094
 F10-ND      -1.10078   0.278717   -3.95  0.000
 M10-D1      0.523061   0.276729    1.89  0.059
 M10-D2     -0.153207   0.279642   -0.55  0.584
 M10-ND      -1.69782   0.294257   -5.77  0.000
 F60-D1      -1.25288   0.309903   -4.04  0.000
 F60-D2      -1.87825   0.312791   -6.00  0.000
 F60-ND      -1.87972   0.272551   -6.90  0.000
 M60-D1      -1.13738   0.303481   -3.75  0.000
 M60-D2      -1.79660   0.317380   -5.66  0.000
 M60-ND      -2.20221   0.278500   -7.91  0.000
 F240-D1     -1.17789   0.311368   -3.78  0.000
 F240-D2     -2.61380   0.329273   -7.94  0.000
 F240-ND     -2.11895   0.275368   -7.69  0.000
 M240-D1     -1.47851   0.312996   -4.72  0.000
 M240-D2     -2.53610   0.327500   -7.74  0.000
 M240-ND     -2.14695   0.274980   -7.81  0.000

Tests for terms with more than 1 degree of freedom
Term     Chi-Square  DF      P
Igroup      205.672  17  0.000

Log-Likelihood = -569.940
Test that all slopes are zero: G = 379.298, DF = 18, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square   DF      P
Pearson       397.181  267  0.000
Deviance      308.682  267  0.040
```

9.5 Combined Male and Female Rat (Parts I and II, 10 Minutes)—Ordinal Response vs logCT, Dcntrst and Rcntrst

[DRS] The following is an investigation of the relative importance of decontamination upon prompt removal from the exposure chamber (Dcntrst) and removal time for decontaminated rats (prompt versus delayed removal from exposure chamber) post-exposure (Rcntrst) on VX toxicity in rats from Parts I and II exposed for 10 minutes. It was found that Rcntrst was not statistically

significant, so it was dropped from the final model fit. Gender by itself was not statistically significant, but its interaction with Dcntrst was found to be statistically significant.

9.5.1 Analysis with Outlier Run

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1         19
           2         50
           3         88
           4         83
           Total    240

Logistic Regression Table

Predictor            Coef    SE Coef       Z      P
Const(1)          14.9923    1.41714   10.58  0.000
Const(2)          16.4799    1.47440   11.18  0.000
Const(3)          18.0857    1.54963   11.67  0.000
logCT            -10.1679   0.885043  -11.49  0.000
Dcntrst           0.631018  0.0972668    6.49  0.000
Gender           0.0614546  0.0760499    0.81  0.419
Dcntrst*Gender    0.253461  0.0910920    2.78  0.005

Log-Likelihood = -214.434
Test that all slopes are zero: G = 177.208, DF = 4, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square   DF      P
Pearson       157.811  107  0.001
Deviance      149.707  107  0.004
```

Variance-Covariance Matrix

Const(1)	Const(2)	Const(3)	logCT	Dcntrst	Gender	DCntrst*Gender
2.00827	2.07487	2.17857	-1.24666	0.0458715	0.0033937	0.0144639
2.07487	2.17386	2.27586	-1.30133	0.0494910	0.0037286	0.0155779
2.17857	2.27586	2.40136	-1.36857	0.0531267	0.0041088	0.0170759
-1.24666	-1.30133	-1.36857	0.78330	-0.0294755	-0.0022638	-0.0096160
0.04587	0.04949	0.05313	-0.02948	0.0094608	-0.0000366	0.0005589
0.00339	0.00373	0.00411	-0.00226	-0.0000366	0.0057836	0.0008197
0.01446	0.01558	0.01708	-0.00962	0.0005589	0.0008197	0.0082977

9.5.2 Analysis without Outlier Run

```
Link Function: Normit

Response Information

Variable      Value  Count
Score[1d]wo   1         19
              2         45
              3         77
              4         79
              Total    220
```

```
Logistic Regression Table

Predictor            Coef    SE Coef       Z      P
Const(1)          17.6610    1.62058   10.90  0.000
Const(2)          19.1773    1.68398   11.39  0.000
Const(3)          21.0145    1.79047   11.74  0.000
logCT            -11.9063    1.02392  -11.63  0.000
Dcntrst           0.886055   0.113573    7.80  0.000
Gender           0.0509879  0.0815494    0.63  0.532
Dcntrst*Gender    0.305413   0.100198    3.05  0.002

Log-Likelihood = -179.637
Test that all slopes are zero: G = 200.115, DF = 4, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square   DF      P
Pearson       124.008  101  0.060
Deviance      119.711  101  0.099
```

Variance-Covariance Matrix

Const(1)	Const(2)	Const(3)	logCT	Dcntrst	Gender	DCntrst*Gender
2.62629	2.71207	2.88115	-1.65065	0.0849353	0.0030739	0.0248829
2.71207	2.83580	3.00333	-1.71978	0.0907566	0.0032316	0.0263864
2.88115	3.00333	3.20579	-1.82974	0.0975141	0.0036866	0.0290668
-1.65065	-1.71978	-1.82974	1.04840	-0.0553832	-0.0019781	-0.0164546
0.08494	0.09076	0.09751	-0.05538	0.0128987	-0.0000985	0.0012399
0.00307	0.00323	0.00369	-0.00198	-0.0000985	0.0066503	0.0000107
0.02488	0.02639	0.02907	-0.01645	0.0012399	0.0000107	0.0100397

9.6 Combined Male and Female Rat (Parts I and II, 60 Minutes)—Ordinal Response vs logCT, Dcntrst and Rcntrst

[DRS] The following is an investigation of the relative importance of decontamination upon prompt removal from the exposure chamber (Dcntrst) and removal time for decontaminated rats (prompt versus delayed removal from exposure chamber) post-exposure (Rcntrst) on VX toxicity in rats from Parts I and II exposed for 60 minutes. It was found that Gender, Rcntrst and their interaction were not statistically significant, so they were dropped from the final fit.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1          6
           2         46
           3         82
           4         66
           Total    200

Logistic Regression Table

Predictor      Coef    SE Coef       Z      P
Const(1)    15.0214    2.16072    6.95  0.000
Const(2)    16.5769    2.19580    7.55  0.000
Const(3)    17.9631    2.23803    8.03  0.000
logCT      -10.9193    1.39863   -7.81  0.000
Dcntrst    0.405996  0.0985633    4.12  0.000
```

```
Log-Likelihood = -195.529
Test that all slopes are zero: G = 78.796, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       41.4541  40  0.407
Deviance      34.9746  40  0.696
```

Variance-Covariance Matrix

```
 Const(1)  Const(2)  Const(3)    logCT     Dcntrst
 4.66870   4.72227   4.81002  -3.00864   0.0266954
 4.72227   4.82153   4.90642  -3.06745   0.0290574
 4.81002   4.90642   5.00877  -3.12668   0.0311403
-3.00864  -3.06745  -3.12668   1.95616  -0.0176269
 0.02670   0.02906   0.03114  -0.01763   0.0097147
```

9.7 Combined Male and Female Rat (Parts I and II, 240 Minutes)—Ordinal Response vs logCT, Dcntrst and Rcntrst

[DRS] The following is an investigation of the relative importance of decontamination upon prompt removal from the exposure chamber (Dcntrst) and removal time for decontaminated rats (prompt versus delayed removal from exposure chamber) post-exposure (Rcntrst) on VX toxicity in rats from Parts I and II exposed for 240 minutes. It was found that Dcntrst, Gender and their interaction were not statistically significant, so they were dropped from the final model fit.

```
Link Function: Normit

Response Information

Variable   Value  Count
Score[1d]  1         13
           2         31
           3         72
           4         83
           Total    199

Logistic Regression Table

Predictor      Coef    SE Coef       Z      P
Const(1)    20.4695    2.58515    7.92  0.000
Const(2)    21.4870    2.60940    8.23  0.000
Const(3)    22.8413    2.65717    8.60  0.000
logCT      -14.3074    1.67986   -8.52  0.000
Rcntrst    0.563102   0.115780    4.86  0.000

Log-Likelihood = -189.356
Test that all slopes are zero: G = 99.061, DF = 2, P-Value = 0.000

Goodness-of-Fit Tests

Method     Chi-Square  DF      P
Pearson       89.7898  40  0.000
Deviance      87.3027  40  0.000
```

Variance-Covariance Matrix

Const(1)	Const(2)	Const(3)	logCT	Rcntrst
6.68301	6.73268	6.85149	-4.33382	0.0324273
6.73268	6.80899	6.92492	-4.37919	0.0351413
6.85149	6.92492	7.06053	-4.46039	0.0383519
-4.33382	-4.37919	-4.46039	2.82193	-0.0235706
0.03243	0.03514	0.03835	-0.02357	0.0134051

DEPARTMENT OF THE ARMY
CDR USARDECOM
ATTN AMSRD CII
5183 BLACKHAWK ROAD
APG MD 21010-5424

OFFICIAL BUSINESS

FIRST CLASS